

Fabrication with Laser-Powder Bed Fusion and characterisation of Zr702 lattices for an orthopaedic application

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy (PhD)

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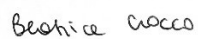
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A handwritten signature in black ink, reading "Beatrice Woco". The signature is written in a cursive style with a horizontal line underneath.

“τὸν φρονεῖν βροτοὺς ὁδώ-
σαντα, τὸν πάθει μάθος
θέντα κυρίως ἔχειν.”

Αἰσχύλος, Ἀγαμέμνων 176-178

“[Zeus] puts mortals on
the journey of comprehension.
And made this the powerful law:
we learn by suffering.”

Aeschylus, Agamemnon 176-178

“I did what I had to do
I saw it through without exemption
I planned each charted course
each careful step along the byway
And more, much more than this
I did it my way”

Frank Sinatra, My way

Abstract

Laser Powder Bed Fusion is an additive manufacturing technology that is industrially exploited for the production of orthopaedic implants exhibiting lattice features, that are open and geometrically ordered porous structures.

In order to ensure the correct functioning of the prostheses, lattices must be made of biocompatible materials, they should exhibit adequate geometric accuracy and structural integrity, and their surface topography should promote (or at least not hinder) the integration of the implant in the body (osteointegration). Nevertheless, the lack of standards and established methods for the measurement and characterisation of the lattices' geometrical accuracy and surface topography currently prevents the full assessment of such products, curtailing the confidence (thus, the market) over them.

This research is devoted to the Laser Powder Bed Fusion manufacturing of octet lattices made in Zirconium (Zr702), a material that is often neglected in the biomedical research, although it offers excellent biocompatibility.

The study is aimed, first, at optimising of the additive manufacturing parameters for the printing of orthopaedic-relevant zirconium octet lattices. Second, it investigates the lattices fabricated with a set of optimal process parameters in a bio-mechanical perspective. Third, this work is aimed at developing methods that allow for the lattice's geometrical and topographical characterisation and that are relevant to orthopaedic implants.

The study identifies a set of criteria that lattice structures should meet to ensure the "adequate geometrical accuracy and structural integrity" for an orthopaedic application. It shows the key process parameters and settings (laser power, scanning speed, printing strategy) that affect the geometrical accuracy and structural integrity of Laser Powder Bed Fusion octet lattices, and provides several sets of manufacturing conditions that meet the above-mentioned criteria on geometry and structural integrity.

This work assesses the quasi-static mechanical properties (particularly the stiffness) of optimised zirconium octet lattices, concluding that the fabricated lattices are relevant to orthopaedics and might be used to substitute compact bones. The biological assessment performed in this study investigates the behaviour of osteoblast-like cells on zirconium lattices and on full bulk disks, concluding that the material is indeed biocompatible, however lattice structures seem to be less biocompatible due to their surface topographies. Chemical or electro-chemical surface treatments are recommended to smoothen the lattices' surface topography and maintain the material's biocompatibility. The study encompasses the measurement and characterization of the lattices' geometry, which was performed by X-rays Computed Tomography, image segmentation and analysis. A key output is the algorithm for the automatic geometrical characterisation of lattice struts and nodes (the key elements of lattice structures) along the different orientations of interest.

Finally, the work entails the measurement and characterization of lattices' surface topography by Focus Variation Microscopy and the development of an algorithm that is relevant to orthopaedic lattice structures. A key output is the characterisation protocol that allows to detect and characterize the Partially Melted Particles, that are a common feature of surfaces generated by Laser Powder Bed Fusion.

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Chapter 1

Introduction

This chapter serves as a foundation of the research study and provides the context in which this thesis was carried out. It outlines the research problem, the attained objectives, addresses the limitations of the research and highlights the contribution to knowledge of the PhD thesis.

1.1 Background of the research

Laser Powder Bed Fusion (L-PBF) is an *additive manufacturing* technology that enables the fabrication of free-form and complex metallic components by melting powder material layer-by-layer. In the biomedical field, L-PBF enabled a re-think and renewal of the design of *orthopaedic implants* (i.e., hip acetabular cups, femoral stems, spinal disks, etc.) and unlocked the possibility to manufacture personalised implants [4].

Load-bearing orthopaedic implants can be produced with a wide range of metallic materials: titanium alloys, stainless steel, CoCr alloys, tantalum, etc. [5]. Ti6Al4V is still considered the “gold standard” for orthopaedics (although originally being developed for the aerospace sector) and is the subject of extensive research in the field of additive manufacturing for biomedical applications. However, its main alloying elements – Aluminium and Vanadium – are causes for concern for the long-term residence in the body and there is increasing interest in developing new alloys for the orthopaedic application [6].

Whilst much work is devoted to new titanium alloys, very limited attention has been given to *zirconium*. Often overlooked in the biomedical field (including academic research), zirconium is biocompatible, processable by L-PBF and offers several advantages over Ti6Al4V [7]. First of all, zirconium is free from sensitizer and cytotoxic elements such as

aluminium, vanadium or nickel, resulting in an improved biocompatibility. Second, zirconium offers a lower elastic modulus compared to Ti6Al4V and, therefore, a reduced risk of “stress shielding”, a condition that affects fully dense metallic orthopaedic implants and that results in the loosening of the prostheses for the patient. Third, zirconium has lower magnetic susceptibility than Ti6Al4V, reducing magnetic resonance image artifacts in patients with metallic orthopaedic implants.

Regardless of the material used, L-PBF is industrially exploited to produce *porous* metallic orthopaedic implants. *Lattice structures*, which are open and geometrically ordered porous structures made by struts connected by nodes, are increasingly adopted in commercial L-PBF manufactured prostheses. Their use is driven by 1) an improved bio-mechanical integration with the body (*osteointegration*) [8]; 2) a significant reduction in material usage, with consequent reduction in the prosthesis’ weight and environmental impact; 3) an increased productivity (manufacturing lattices is faster than full objects) [9]; and 4) the opportunity to make patient-specific implants.

Octet lattices offer high specific strength and specific stiffness and are therefore well-suited for load-bearing orthopaedic implants [10]. Furthermore, clinical studies have shown that octets support the bone ingrowth, which is a key factor for the implementation of such structures in orthopaedic implants [10].

Lattices should exhibit an adequate *geometric accuracy* with respect to the designed structure and *structural integrity* to ensure the correct functioning of the orthopaedic implant [10]. In addition to this, their *surface topography* should promote (or at least not hinder) the osteointegration [11].

However, the geometry and surface topography of lattice features (and porous features, in general) are not normed, with the result that lattices embedded in commercial implants are not measured, characterised or assessed in the industry [12].

The lack of standards on these elements are (also) due to:

- 1) The significantly challenging technological issues for the measurement of both the geometry and the surface topography of lattice structures.
- 2) The lack of established methods for the characterization of the geometry of complex-shaped objects like the lattice structure.
- 3) The lack of established methods for the characterization of free-from surfaces generated by L-PBF, particularly for surfaces aimed at the osteointegration.

Being able to measure and characterise geometry and topography of L-PBF-manufactured lattices that are aimed for an orthopaedic application – especially at an industrial level – would allow to assess the process-and-product's quality and to increase the confidence over these relatively new implant's features.

1.2 Aim and objectives of the study

This research is devoted to the investigation of Zr702 (commercially pure Zirconium) octet lattice structures fabricated by L-PBF.

Zr702 can be processed by L-PBF and was recently re-proposed for the production of biomedical implants by L-PBF [7]. However, to the best of the author's knowledge, this material has not been employed in the fabrication of lattice structures. Fabricating lattices with good geometric accuracy and structural integrity requires extensive L-PBF process knowledge, which is unfortunately lacking in the case of zirconium. This leads to the first research question:

- *“How can Zr702 be processed by L-PBF to fabricate octet lattice structures for an orthopaedic application?”*

The second research question arises from the lack of knowledge on the bio-mechanical performance of Zr702 lattice structures:

- *“How do L-PBF-fabricated Zr702 octet lattice structures behave in a bio-mechanical perspective?”*

The last research question arose based on the following considerations. Industry is already exploiting L-PBF for the manufacturing of orthopaedic implants with porous features and

lattice structures, and whilst traditional fully-dense orthopaedic implants are subjected to strict norms on geometry and surface topography, porous features (including lattice structures) are not. Nevertheless, it is precisely the geometric accuracy and surface topography of those features that are crucial for the clinical success of orthopaedic implants. At the current stage, an important step in the quality control of these most recent medical devices is missing. There is indeed a clear gap in the international standards that regulate additively-manufactured orthopaedic prostheses [12].

Because of the technical challenges that are encountered in the measurement of the lattice geometry and surface topography, there is today the need to develop effective methods for the quality control of such features embedded in orthopaedic prostheses, as the characterisation methods are not well-established.

From this consideration, the third research question of the thesis is raised:

- “How can L-PBF-fabricated zirconium octet lattice structures be characterised in terms of geometry and surface topography?”

To answer these questions, the author adopted a multi-faceted approach that aims at investigating the L-PBF zirconium lattices under different perspectives. This is the author’s preferred problem-solving strategy as it allows to propose applications-oriented solutions. The assessment of multiple functional aspects that are critical for orthopaedic prostheses (the geometrical accuracy, surface topography, the bio-mechanical behaviour of the fabricated lattices) is part of this work due to this special *modus operandi*.

The methodology adopted in this study will allow to achieve the following objectives:

1. Investigate different L-PBF manufacturing process settings and suggest optimal L-PBF process settings to fabricate Zirconium lattices with suitable geometrical accuracy and structural integrity.
2. Assess the mechanical and biological performances of the structures fabricated with the optimal process settings in relation to the material’s properties.

3. Develop a protocol to characterise the geometry of L-PBF lattice structures with an “orthopaedic-tailored” approach.
4. Develop a protocol to characterise the surface topography of L-PBF lattice structures with an “orthopaedic-tailored” approach.

1.3 Boundaries of the research

The author acknowledges the following limitations to her research work.

First, the optimisation study on L-PBF to fabricate Zr702 octet lattice was performed on a single lattice design, and it was based on two printing strategies: the “island strategy” and the “contour strategy”. These strategies are widely adopted in the L-PBF manufacturing, and the “island strategy” was chosen as first attempt strategy following the work of Aristizabal *et al.* [7] on the L-PBF optimisation of Zr702 bulk structures.

Second, as the access to Zirconium and the L-PBF machine was limited (it was offsite), the experimental study on L-PBF was performed on a limited number of samples. As such, the repeatability of the biological tests might have been influenced.

Third, the mechanical performance of the lattice structures was evaluated by quasi-static mechanical tests to determine their stiffness. The assessment of the fatigue resistance, which is naturally critical for orthopaedic implants, requires a very large sample size and is out of scope for this study.

1.4 Contribution to knowledge

The main contribution to knowledge of this work is represented by:

- 1) The optimisation of L-PBF manufacturing process for the fabrication of Zr702 lattice structures from a bio-mechanical perspective.
- 2) The development of original methods for the geometrical and topographical characterisation of metallic lattice structures fabricated by L-PBF and that are relevant to lattices which are embedded in orthopaedic implants.

Thanks to this work it was possible to:

- 1) Provide L-PBF process knowledge for the manufacturing of Zr702 octet lattice structures.
- 2) Develop and implement an approach for the L-PBF process optimisation tailored to biomedical lattice structures.
- 3) Identify a set of L-PBF process parameters and printing strategy for the fabrication of Zr702 octet lattice structures with characteristics that are suitable for an orthopaedic application, and suggest improvements for the fabrication of Zr702 octet lattice with high architectural porosity.
- 4) Establish the mechanical performances of zirconium lattice structures and provide preliminary results on the biological performance with *in vitro* tests.
- 5) Develop an original algorithm for the geometrical characterisation of metallic lattices that enables the measurement of lattice structural features (struts and nodes) across the 3D space.
- 6) Develop an original method of characterisation of L-PBF lattice surface topographies that is able to describe the geometry and the feature density of Partially Melted Particles.
- 7) To suggest a link between source powder and manufacturing settings to the quality of lattice topographies

1.5 Structure of the thesis

The thesis consists of eight chapters and two appendices.

Aside from this chapter (*Chapter 1 Introduction*) and the concluding chapter (*Chapter 8 Conclusions*), which answers to the research questions outlined above and suggests research directions for future work, the thesis is structured in the following:

- *Chapter 2 Literature review*, which describes the materials used in orthopaedics and the biocompatibility issue, it discusses the role of Ti6Al4V in orthopaedics and explains why apparent little attention has been given zirconium for this application. It illustrates the role of L-PBF in the orthopaedic industry and the

special properties of lattice structures, as well as the challenges encountered in their fabrication by L-PBF. It discusses the challenges and opportunities in the characterization of geometry and surface topography of lattice structures. Finally, it highlights the knowledge gap and reasserts the objectives of the study.

- *Chapter 3 Materials and methods*, which describes the methods and procedures used to respond to the research questions and attain the thesis' objectives.
- *Chapter 4 Zirconium manufacturing by L-PBF and non-destructive characterisation of lattices: Results and Discussion*, which presents the results of the L-PBF process optimisation for the fabrication of Zr702 octet lattice structures and discusses the role of process parameters on the various features of the lattices. It illustrates the results of the geometrical characterization by non-destructive testing and discusses the original method for the automatic measurement of the lattice features.
- *Chapter 5 Mechanical behaviour of L-PBF Zr702 and Zr702 lattice: Results and Discussion*, which presents and discusses the mechanical properties of zirconium fabricated by L-PBF and the mechanical behaviour of the optimal Zr702 octet lattice structure.
- *Chapter 6 Biological performance of Zr702 lattice and bulk material: Results and Discussion*, which presents and discusses the behaviour of the material and the Zr702 lattice structure in the biological environment.
- *Chapter 7 Surface topography of lattices: Results and Discussion*, which illustrates the surface topography and discusses the original method that was developed to detect and characterise the partially melted particles, a typical feature of L-PBF generated surfaces that are a cause of concerns for orthopaedic implants.
- *Appendix I* contains the original MATLAB codes that were developed in this work
- *Appendix II* provides the rationale behind the biological methods chosen for this study.

Chapter 2

Literature Review

As the global life expectancy is increasing, the demand for orthopaedic implant surgery is rising [13]. According to a report by Market Research Future®, the global orthopaedic implants market will grow from \$45.30 billion in 2021 to \$67.48 billion in 2030 [14].

The industry is exploiting a wide range of materials for the production of orthopaedic implants. Nevertheless, metallic materials (notably Ti6Al4V) are still the preferred choice. Additive Manufacturing (AM) of metals -which encloses a variety of processes that fabricate objects by progressively adding material – is a promising technology for the orthopaedic industry and can help expand the market. Some AM technologies – notably those based on powder beds - have been recently applied in this field at an industrial level, leading to revolutionary designs in orthopaedic prostheses and opening to the industrial production of personalised medical devices.

Porous structures – particularly lattices – have been adopted in commercial additively manufactured implants [4], as they offer several advantages over bulk implants, including cutting material usage, better mechanical interlocking with the native bones, and improved osteointegration, with decreased risks of implant failure [8, 15].

In this chapter, the first section (§2.1) will be devoted to the description of orthopaedic implants, the definition of biocompatibility, and the discussion of the materials selection of the fabrication of orthopaedic implants. In §2.2, the role of Ti6Al4V in orthopaedics will be discussed, illustrating both the advantages of this material and its drawbacks, with reference to its long-term biocompatibility. The §2.3 is devoted to Zirconium, the material that was selected for this study. In this section, the author describes the special properties of this metal and explains why apparent little attention has been given to zirconium in orthopaedics. The following section (§2.4) focuses on the AM of metals with particular

attention to the technologies deployed in orthopaedics at the current time by the industry (Powder Bed Fusion technologies), and the implications and opportunities on the design of orthopaedic products will be stressed. Lattice structures (open and geometrically ordered porous structures) will be presented in §2.5, discussing their topology (§2.5.1), mechanical behaviour (§2.5.2), and the constraints that their dimensions need to satisfy to be functional in an orthopaedic application and manufacturable by Laser Powder Bed Fusion (§2.5.3).

In the following section (§2.6), the challenges of the fabrication of lattices by L-PBF will be discussed, illustrating the difficulties of achieving the ideal design geometry, with respect to the strut size, node size, and architectural porosity. There, it will become evident that process optimisation is a critical step in order to achieve lattices of suitable quality for the biomedical application. The need and the challenges of the characterization of lattices for orthopaedic prostheses, as well as the exciting opportunities offered by X-rays Computed Tomography (X-CT), a non-destructive test (NDT) that enables the characterization of these complex structures, will be discussed in §2.7. Finally, section §2.8 discusses the role of surface topography as a bridge between the manufacturing of the lattice structures and the performance of the orthopaedic implants (§2.8.1). Subsection §2.8.2 illustrates the features of the surface topography of L-PBF manufactured objects and highlights the challenges encountered in their characterisation. Subsection §2.8.3 addresses the controversy on the beneficial/harmful role of partially melted particles (PMPs) that populate the surfaces generated by AM, whilst the challenges and opportunities in their characterisation are discussed in §2.8.4. Subsection §2.8.5, which closes this literature review, is dedicated to the density of the PMPs as a possible mean to characterize L-PBF lattice structures. Finally, the §2.9 summarises the key findings of the literature review, highlights the knowledge gap in the existing literature and reasserts the aim and objectives of this research work.

2.1 Materials for orthopaedic implants

Orthopaedic prostheses are biomedical implants aimed at restoring the form and function of diseased or missing bones through their bio-mechanical integration with the body (osseointegration) [13]. The implants need to bear loads and repeated stresses [16, 17], support the growth of newly born bone tissue on its surfaces [17] and ensure a life expectancy of about ten-twenty years, depending on the type of the implant [5].

As such, prostheses need to be manufactured with materials that offer adequate mechanical strength and fatigue resistance, and appropriate durability in the body. Moreover, they need to be *biocompatible*.

The definition of biocompatibility has changed and adapted through the years [5]. Whilst many embrace William's definition of biocompatibility as the quality of a *material* to integrate into a biological system without triggering local or systemic effects [18, 19], the author prefers the definition introduced by Becker which sets biocompatibility as "the capability of a *prosthesis* implanted in the body to exist in harmony with tissue without causing deleterious changes" [20]. The latter definition acknowledges the fact that the integration and functionality (and therefore the success or failure) of an implant in a host body depends not only on the bio-chemical compatibility of the prosthesis material(s) – as William's definition would suggest – but also on a series of properties of the medical device, including its design, load-bearing capacity and surface finish [21].

Becker's perspective on biocompatibility is pragmatic and yet comprehensive. Rather unsurprisingly, regulatory bodies such as the U.S. Food and Drug Administration (FDA) and ISO adopt the same approach, as they are engaged in regulating and assessing the biocompatibility of the entire prosthesis, rather than making prescriptions on single materials [5].

In this study, a "biocompatible material" (or *biomaterial*) is a synthetic or natural material that is suitable for a long-term use in biological environments as it provokes no adverse reactions to the host body and does not degrade (*biostable*) [5]. It follows that

biodegradable and resorbable biomaterials (like Magnesium [22]) are excluded from this investigation.

Orthopaedic implants are continuously innovated as new biomaterials become available and manufacturing technologies are developed. Furthermore, the material selection depends on the function of the prosthesis features and is continuously reviewed in the light of new clinical evidence.

It is perhaps unsurprising that implants have been manufactured with a whole different range of materials in history. The first known orthopaedic prosthesis - a false toe of a female mummy of Ancient Egypt - was made of wood and leather [5, 23]. Today, the orthopaedic prostheses are commonly made with metals (e.g. commercially pure Titanium (CP-Ti), Ti6Al4V and Ti6Al4V ELI¹, stainless steel, Co-Cr-Mo alloys, and, recently, tantalum and zirconium) [5, 24], although polymers (PMMA², UHMWPE³, and PEEK⁴) [16, 25] and ceramics (e.g. alumina and zirconia) [5] are employed as well.

Metallic biomaterials are often the favourite choice for orthopaedic implants, as they offer high mechanical properties (including high strength, fracture toughness, hardness, tenacity, ductility, and fatigue resistance) and relatively high corrosion resistance in biological fluids, to which they owe their biocompatibility [16]. However, metals exhibit higher densities and elastic moduli compared with bones, which result in heavier and stiffer bone replacements. The clear difference in the elastic moduli of metallic biomaterials (Titanium, Ti6Al4V, Stainless Steel, CoCr, Tantalum) and bones can be appreciated in Figure 1. The stiffness mismatch between the metallic materials and the natural bones is particularly problematic for orthopaedic implants, because it is deemed to cause the “stress shielding”, a condition that leads to the loss of the prostheses (this issue will be further discussed in section §2.6.2).

¹ Ti6Al4V ELI is Titanium Grade 23, where ELI stands for Extra Low Interstitials

² PMMA stands for polymethylmethacrylate.

³ UHMWPE stands for ultra-high-molecular-weight polyethylene.

⁴ PEEK stands for polyetheretherketone

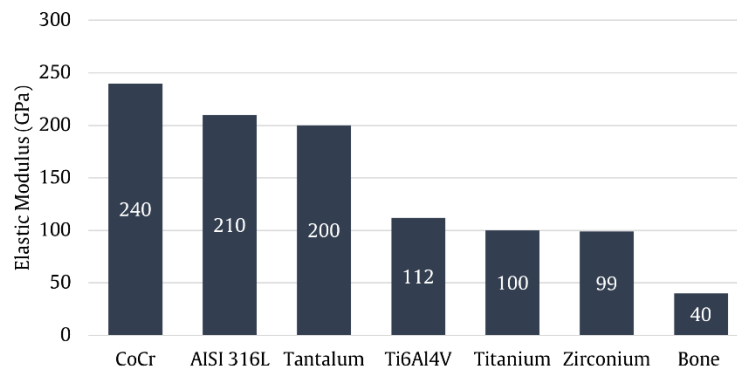


Figure 1 Elastic moduli of common metallic materials deployed in the manufacturing of orthopaedic prostheses and the original bones [13].

2.2 Ti6Al4V: the aerospace material that became the “Gold standard” for orthopaedic implants

Ti6Al4V is widely exploited for the fabrication of implants for orthopaedic replacement (e.g. hip, knee, shoulder, spine, elbow and wrist) as well as for bone fixation (e.g. screws and plates). Ti6Al4V is characterised by high specific strength (strength per density) and offers good biocompatibility [24], which is related to its corrosion resistance in the biological environment. The passivating oxide layer (TiO_2) that covers its surface makes the material biostable and *bioinert*, as it does not actively interact with the biological environment, and it is not able to induce the formation of apatite layers onto its surfaces (*osteointductivity*).

Ti6Al4V is typically regarded as the “gold standard” against which evaluating other biomaterials for orthopaedics. However, this material was developed for the aerospace industry [6] rather than for the biomedical sector, and there are some concerns over its prolonged use in the human body [6]. Its main alloying elements, Vanadium and Aluminium, are indeed associated with negative reactions: Vanadium is reported to have cytotoxic effects [6], whilst Aluminium is suspected to be involved in neurotoxicity and neurodegenerative diseases [6] and can cause allergies [26]. This is particularly worrisome for young patients, and many are working in the development of new titanium-based

alloys with other elements, such as Zirconium (Zr), Tantalum (Ta), Niobium (Nb), Molybdenum (Mo), and tin (Sn) [6, 26-31].

2.3 Zirconium as biomaterial?

Zirconium (from the two Persian words “gold” (*zar*) and “colour” (*gun*) [32]) is a refractory metal that exhibits chemical properties similar to those of Titanium. Indeed, the two metals belong to the same group in the periodic table.

Zirconium offers an exceptional corrosion resistance to several agents including acids, alkalis, and brine, thanks to the autogenous thin oxide layer (ZrO_2) that covers its surface. It is precisely this oxide that makes zirconium biocompatible (as it is the case for titanium), and no local or systemic toxic effects have been reported [33, 34]. Indeed, it is used as an alloying agent in steel for surgical appliances and, as mentioned earlier in §2.2, in newly developed titanium-based biomedical alloys [6, 35-37]. Furthermore, zirconia (ZrO_2), is commonly used in commercial ceramic orthopaedic implants [32].

Nevertheless, pure Zirconium (as well as its alloys) is rarely exploited or proposed for the fabrication of implants [33]. In fact, even academic research overlooks it in favour of the “classic” Ti6Al4V or other titanium-based alloys, with very limited literature promoting for its use [24, 38, 39].

Hunter *et al.* [34] report that zirconium had been actually exploited in the '50s (ten years before the introduction of Ti6Al4V) for orthopaedic screws and bone plates - with promising results - in England. This was a time of great development for orthopaedics (the World Wars had left many without limbs). At the same time, however, governments were pushing for nuclear power programmes: zirconium was soon redirected to the nuclear market and became temporarily unavailable for other industries, including the biomedical sector.

Zirconium has, indeed, special properties that made it an “international nuclear material” [40]: it exhibits a low absorption cross section for neutrons (about thirty times lower than that offered by Ti) [24, 41], that determines its high resistance to neutron absorption

(critical for safety in nuclear plants). It is therefore unsurprising that 90% of zirconium is currently applied in the nuclear sector, where it is exploited for the nuclear reactor tubing [40].

When, in 1958, Zirconium became available again outside the nuclear industry, titanium and its alloys had already asserted themselves as biomaterials in orthopaedics, and there was no interest in investing on zirconium for medical application [34]: the good biocompatibility of zirconium was rapidly forgotten or neglected.

Nevertheless, in 2018, Mehjabeen *et al.* [24] claimed that there was a growing attention towards the use of zirconium and its alloys for orthopaedics and dental applications.

Between 1997 and 2004, a zirconium alloy known as “Oxinium” has been used for femoral components and femoral heads in over 65,000 surgical operations [42]. Oxinium was specifically developed for orthopaedic application to overcome the drawbacks of Co-Cr alloys, notably their tribological behaviour and biocompatibility issues. The base material was Zr-2.5%Nb – which had been developed by the USSR for nuclear applications [40] – and the prostheses were heat-treated at 500 °C in air to oxidise their surfaces, to create a zirconia layer for increased wear resistance [42].

More recently (2020), commercially pure zirconium (Zr702) disks fabricated by Additive Manufacturing (a disrupting technology that will be presented in detail in the sections §2.4 and §2.5, and hereafter referred to as AM) were tested *in vitro*, confirming the good biocompatibility of the material in its pure form.

Since then, however, no article has appeared on commercially pure zirconium for the orthopaedic application. In fact, a search conducted on Scopus⁵ resulted in fourteen journal papers on zirconium-based materials for orthopaedics. Ten [37, 43-51] are relevant to titanium alloys (in which Zr is used as an alloying agent), three [52-54] deal with metallic glass (which is a type of intermetallic alloy), and one is on Mg alloys [55].

⁵ The search was conducted in Scopus (www.scopus.com) with the following criteria: 1) written in English; 2) article title, abstract, or keywords containing Zirconium AND NOT (« zirconium dioxide » OR « zirconia » OR ceramic OR biodegradable OR bioresorbable) AND orthopaedic; 3) published since 2021.

Hence, only a narrow community of researchers seems to be aware of the biocompatibility of zirconium and its behaviour in the biological environment, in general. Therefore, a review of the existing literature on this topic is timely.

As mentioned above, zirconium owes its biocompatibility to the oxide layer (zirconia) that covers its surfaces. The superficial autogenous layer makes the material “bioinert”: the metal is indeed stable in the biological environment and it is incapable of promoting bone formation at an early stage after implantation [39, 56, 57].

As the same is valid for titanium and its alloys, it is worth understanding how the two materials compare in terms of biocompatibility.

Sollazzo *et al.* [58] reported that zirconia-coated titanium implants led to a higher bone-to-implant contact than commercially pure titanium ones at four weeks after implantation in rabbits. AlFarraj *et al.* [33] concluded that zirconium (Zr702) and commercially pure titanium had comparable bone healing response (always in terms of bone-to-implant contact) in rabbits, after eight weeks of implantation. Interestingly, they observed that there was no advantage in modifying the materials with hydroxyapatite. The latter is the main inorganic component of bones and is often exploited - at least at research levels - for coatings and superficial treatments of biomedical scaffolds to turn bioinert materials into *bioactive* ones [59-61]. The authors supposed their results might have been affected by the observation period chosen in the study, which is relatively long compared to the healing period of rabbits (four weeks [58]), and suggested that the surface modification with hydroxyapatite might actually play a role in the initial stages of the healing process. In the author's view, these results indicate that pristine commercially pure zirconium Zr702 – albeit bioinert rather than bioactive – is suitable for orthopaedic applications and can offer comparable or even better clinical outcomes than titanium and its alloys.

Liu *et al.* [62] concluded that zirconium exhibits good biocompatibility both in bulk and in powder forms (average size of 44 μm , which is in the same range of metallic powders used in AM), whilst titanium and niobium powders are slightly cytotoxic.

Interestingly, Mehjabeen *et al.* [24] reports that zirconia is more resistant to bacterial adhesion than titanium or TiO_2 . As such, zirconium implants would be less prone to be colonised by bacteria, with reduced risks of implant failure due to bacterial infections (the reader who is interested in the topic is referred to the reviews by Campoccia *et al.* [63] and Abdallah *et al.* [64]).

In addition to these advantages, zirconium has lower magnetic susceptibility than titanium (about 50% lower), meaning that quality of magnetic resonance imaging of implanted prostheses would be improved [24]. Furthermore, the elastic modulus of this material is 80-99 GPa [7, 65], which is lower than Ti4Al6V (refer to Figure 1).

Overall, experimental works that investigated the biocompatibility of Zr and its behaviour in comparison with other biomaterials encourage to explore and use zirconium for biomedical applications. On the other hand, the large stiffness mismatch with the native bones calls for the development of solutions that aim at reducing the elastic moduli of zirconium orthopaedic prostheses. Such solutions are currently represented by porous metallic structures and can be fabricated by AM, that is the focus of the following section.

2.4 Laser-Powder Bed Fusion AM for metallic porous orthopaedic implants

AM is “the process of joining materials to make parts from the data of 3D model, usually layer upon layer, as opposed to subtractive manufacturing and formative manufacturing technologies” [66].

AM offers unprecedented design freedom with its capability of creating complex geometries, almost free-form surfaces, and – for some processes – porous features. Moreover, it represents an economically sustainable way to manufacture small batches (from one to a few units) of products, *de facto* enabling for product customisation.

These aspects alone explain the prompt adoption of AM by the industry for orthopaedics, which has been driven by 1) the possibility to manufacture patient-specific medical devices (key for personalised medicine) at viable costs; and 2) the promise to improve the

osteointegration of orthopaedic implants by the reduction of the stiffness mismatch with the native bones, which is achieved through the introduction of porous structures. Such features are increasingly adopted in the design of commercial acetabular cups, femoral stems, spine disks, etc. [67].

It is therefore unsurprising that the most widely exploited AM technologies in the biomedical industry are the Powder Bed Fusion (PBF) systems, as they enable for the fabrication of porous features. With these processes, the metallic powder is deposited in layers to form a powder bed, and the material is selectively melted by a laser or electron beam (in the Laser PBF or the Electron Beam PBF, respectively) to generate an object according to a CAD design, layer-by-layer. The technology is illustrated in Figure 2.

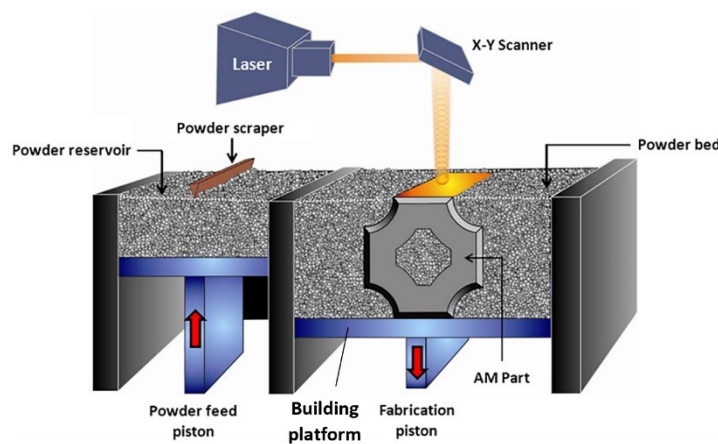


Figure 2 Illustration of the L-PBF system. AM part stands for Additively Manufactured part. Notice that the AM part is generated on the “building platform”. Image adapted from [3].

The Laser Powder Bed Fusion (L-PBF), often referred to as “Selective Laser Melting” (SLM), can process a wide range of biomaterials, from titanium and its alloys to stainless steel (notably 316L), from CrMo alloys to zirconium [7, 68]. Extensive literature reviews [69] already exist on the subject and the author will not attempt to repeat the same exercise here.

Several researchers have focused on the development of titanium-based porous structures by L-PBF [70-75]. However, no previous work on commercially pure zirconium has been reported. As such, the author explored the potential use of Zr702 for the L-PBF fabrication

of lattice structures, that are open and geometrically ordered porous structures (these will be illustrated in detail in the following sections).

This study moves from the work of Aristizabal *et al.* [7], who investigated the processability of Zr702 with L-PBF and confirmed the biocompatibility of the bulk (fully dense) disks made with the same technology.

2.5 Lattice structures in orthopaedic implants

In this section, lattice structures will be described and discussed in the context of orthopaedics. In §2.5.1, the advantages offered over bulk structures in the context of orthopaedic prosthetics will be enumerated, and the choice of the octet lattice (that is the selected topology in this study) will be explained. The mechanical behaviour of lattices will be discussed later in subsection §2.5.2. There, the impact of the structure design on its mechanical behaviour will be shown by comparing the mechanical properties of lattices with those of other open porous structures and with the materials' properties. Furthermore, norms on mechanical testing of porous structure will be described. Finally, in §2.5.3, the dimensions of lattices for orthopaedics will be discussed in relation to the lattice functionality and manufacturability by L-PBF, and a design space for the octet lattice will be introduced.

2.5.1 Lattice structures

Lattice structures are geometrically ordered, open porous structures made by *struts* (or beams) connected by nodes. These structures are made by the repetition of a *unit cell* in the three dimensions. Examples of lattice topologies are given in Figure 3.

The porosity phase in an ideal lattice structure is interconnected and will be called hereafter “architectural porosity”.

As mentioned earlier, the use of lattices seems particularly promising for biomedical applications. Indeed, their generally high specific surface provides an increased

mechanical interlocking between the implant and the surrounding biological tissues compared to fully dense implants [10], ideally leading to a faster osteointegration.

Furthermore, the open porous structures enable for the 3D colonization of the prosthetic device, the tissue ingrowth and possibly allows to develop a vascular system within the prosthesis [8] or to deliver drugs *in situ* [15].

Importantly, lattice structures exhibit lower stiffness than the material with which they are made. The mechanical behaviour of these structures is perhaps their most fascinating characteristic and will be discussed in detail in the next section (§2.5.2). Here, the author would like to highlight that lattice structures can be finely tuned to target specific elastic moduli like the natural bones', which should reduce the risk of stress-shielding. In addition to these advantages, lattices offer a faster production of products in AM as well as reduced consumption of material and lightweighting [9].

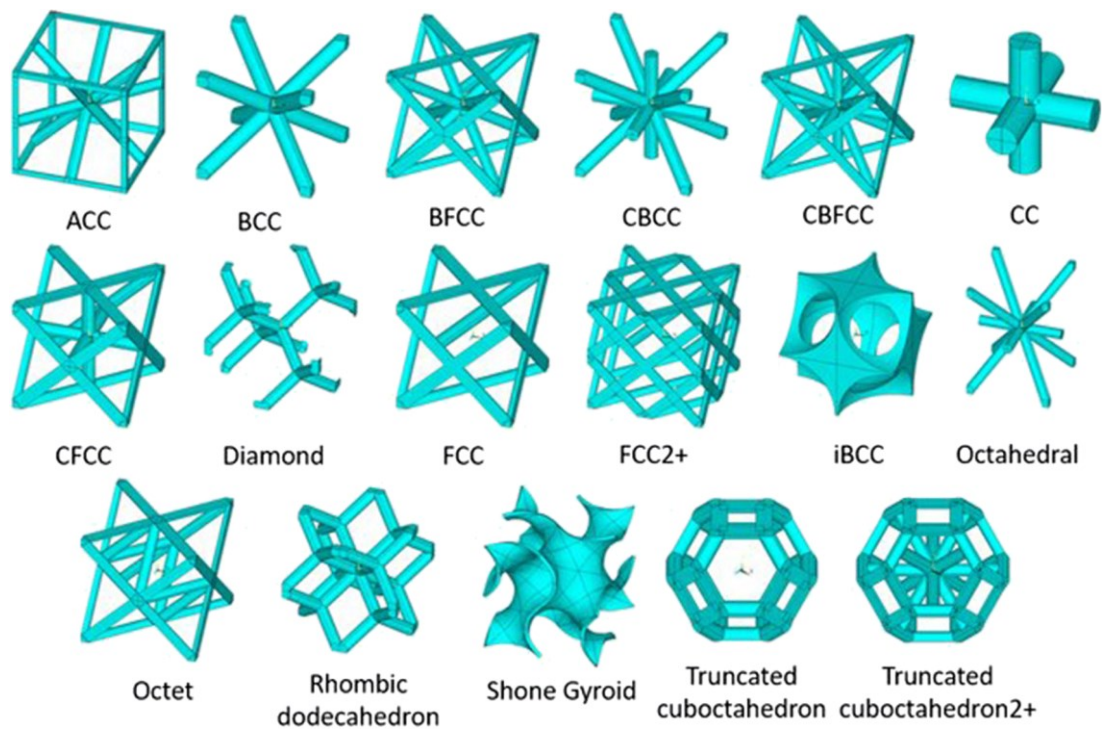


Figure 3 Examples of lattice topologies that can be manufactured by L-PBF [67]. Notice that iBCC and shone gyroid are surface-based (not strut-based) and they belong to triply periodic minimal surface structures. Original image from [76].

The *octet* (depicted in Figure 4a) was selected as geometry lattice for this study, in virtue of the demonstrated favourable clinical outcomes [10, 77]. In this regard, this study moves

from the extensive work performed by Pasini and colleagues on the fabrication of Ti6Al4V octets by L-PBF [10, 72, 77, 78].

The octet topology may be described as the union of an octahedron (the inner structure) and eight tetrahedrons [79], although it might be visualised more easily by thinking of a Face-Centred Cube (FCC) crystallographic structure [70]. It is also a Platonic solid, which means that all its struts have the same shape and length L [79] (see Figure 4a).

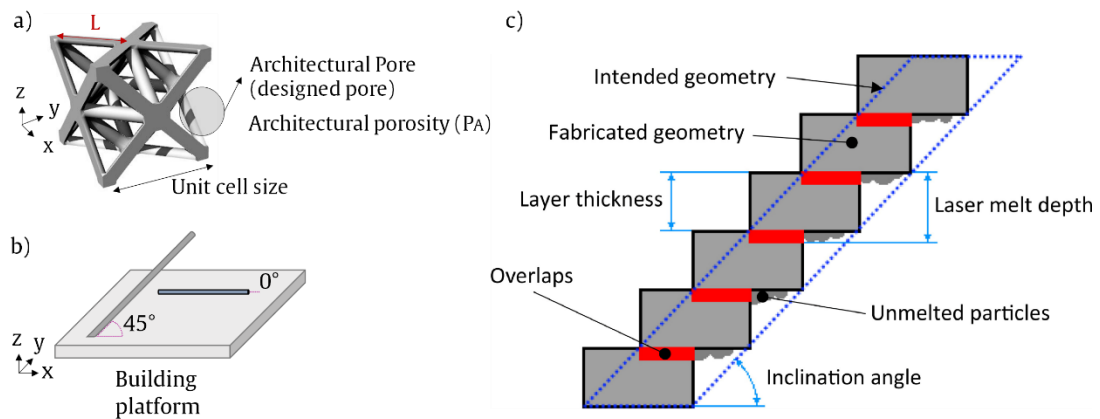


Figure 4 a) Octet topology, the chosen lattice design for this experimental study; b) highlight on the lattice strut orientation in the octet topology: horizontal and diagonal struts with respect to the building platform; c) scheme that illustrates the generation of a lattice strut with a 45° inclination angle from the building platform [80].

It is worth noting here that the orientation of the beams in the lattice (Figure 4b) is parallel to the building direction (horizontal struts) or inclined by 45° with respect to the building platform (hereafter called diagonal struts). In the L-PBF, the diagonal struts are generated by melting subsequent powder layers like a staircase, in which each step is built over a portion of solidified material (overlaps, in Figure 4c), and on unmolten powder bed. The stair-case effect that derives from the manufacturing constitutes a typical geometrical defect of L-PBF inclined objects Figure 4c.

2.5.2 The mechanical behaviour of lattices: theoretical models and experimental behaviour

The mechanical behaviour of lattice structures is one of the most crucial features that drove the adoption of such structures in load-bearing orthopaedic prostheses [8, 81].

From a mechanical perspective, lattices are composite materials made by two phases: material and air. It follows that (metallic) lattice structures exhibit a lower stiffness compared to the (metallic) material and a high specific strength. The combination of these characteristics makes these structures suitable for matching the (mechanical) characteristics of the native bones which the prosthesis should substitute [82]. In particular, matching the prosthesis stiffness with the bone elastic moduli is deemed to be necessary and critical for the osseointegration of the implant in patients, as it allows a physiological redistribution of the mechanical stresses between the prosthesis and the surrounding tissues [82]. The mechanical stiffness match reduces the risk of the so-called “*stress shielding*”, a condition where the stresses are mainly bore by the stiffest part in the system (for example, a fully dense metallic prosthesis) whilst the soft parts (typically, the surrounding bones which are in mechanical contact with the prosthesis) are shielded [81, 83]. Because the bone is a mechano-transducer tissue, it resorbs (decomposes) in absence of adequate mechanical stress, like in stress shielding conditions. This situation is unfortunately often encountered when fully dense metallic prostheses are employed, and it is caused by the great mismatch between the elastic moduli of the materials (above 100 GPa for Ti6Al4V, Ti, stainless steel, etc.) and of the native bones (0.5–40 GPa considering cancellous and compact bones altogether). Stress shielding (among other factors such as wear [84]) can lead to the “aseptic loosening” of the prosthesis, a condition in which the implant fails in absence of infection (aseptic) due to the loss of fixation, with consequent risks of new operations for patients and rise of healthcare costs.

The mechanical behaviour of lattices depends on the material with which the lattice is made, on the fraction of material over the lattice unit cell (also known as relative density, ρ_{rel} , and on the geometry of the lattice (lattice type or topology).

It is worth stressing that the lattice mechanical properties are not intrinsic properties (material properties), but rather properties of the structure. Because of their composite

nature, the stiffness of lattices are *not* referred to as “elastic modulus” or “Young’s modulus”, which are indeed specific to a material.

However, it is common to express the lattice mechanical properties (such as stiffness and strength) as a function of the fraction of the property of the parent material with which the lattice is made. The “Gibson-Ashby” model is the most widely adopted model to express and predict the strength and stiffness of lattice structures [85, 86]. In the case of stiffness, the lattice performance depends on the relative density (ρ^*), the Young’s modulus (E_0), a numerical coefficient (C_1), and a power coefficient (n), as shown in Equation 1.

Equation 1

$$\frac{E}{E_0} = C_1 \left(\frac{\rho}{\rho_0} \right)^n = C_1 \times (\rho^*)^n$$

In the case of strength, the lattice performance depends on the relative density (ρ^*), the yield strength, a numerical parameter (C_2) and a power coefficient (m), as shown in Equation 2.

Equation 2

$$\frac{\sigma_Y}{\sigma_{Y,0}} = C_2 \left(\frac{\rho}{\rho_0} \right)^m = C_2 \times (\rho^*)^m$$

The n and m coefficients in Equation 1 and Equation 2, respectively, define the shape of the function. The specific shape of the stiffness (or strength) function with the parent materials depends on the topology of lattice structure. There are two distinct functions which describe the mechanical behaviours of *stretch-dominated structures* and *bending-dominated structures*, respectively.

The first class (stretch-dominated structures) encompasses the lattice topologies in which the struts (or beams) are axially loaded, developing tensile or compressive stresses; the second class (bending-dominated structures) is characterised by struts that are subjected to bending moments when the lattice structure is compressed [80]. Figure 5a illustrates the different stress distribution in the two types of structures at the unit cell level.

For stretching-dominated topologies, the coefficients n and m are both equal to 1, whereas for bending-dominated topologies, the coefficients are equal to 2 and $2/3$, respectively [80, 86-90].

It follows that stretched-dominated structures, also called micro-trusses, are characterised by higher specific strength and stiffness than bending-dominated structures with the same relative density. Stretch-dominated structures are indeed the preferred solution for light-weighting applications. Instead, bending-dominated structures are more compliant and deform consistently by a “mostly uniform collapse” [86], and they are more suitable for energy-absorption applications [80, 87].

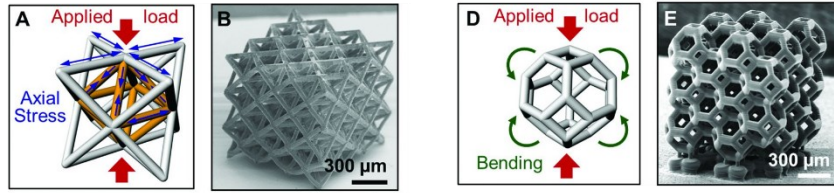
The mechanical behaviours of stretching-dominated and bending-dominated structures under compression loads are shown in Figure 5b.

In general, the stress-strain curves of the two structures exhibit three stages: 1) the linear elastic region, which terminates with the struts yielding; 2) a stress plateau, in which cells collapse by buckling, brittle fracture or yielding, depending on the lattice topology and material; 3) the densification region, in which the structure is progressively compacted as cells start packing one against each other.

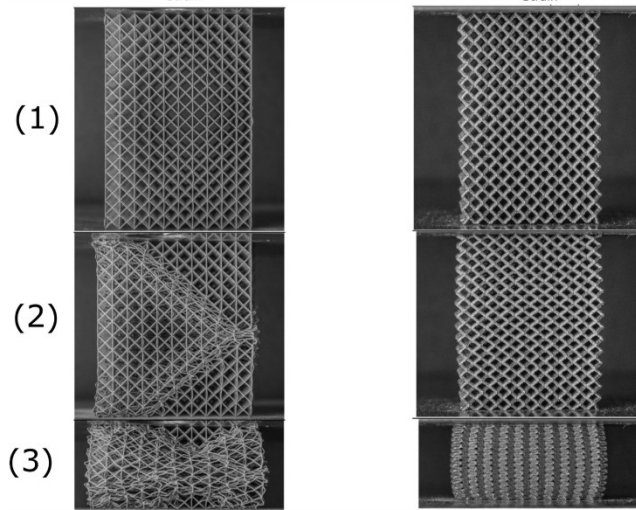
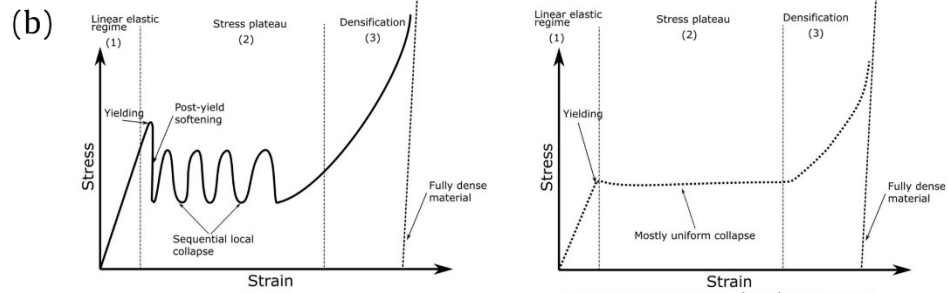
The main difference between the two stress-strain curves is given by the peculiar failure of the cells in the second stage (stress plateau): the stretching-dominated structures undergo consecutive local fractures, which lead to the oscillation of the stresses around a mean value called the plateau stress. Instead, the bending-dominated structures are characterized by a progressive buckling of the structure, in which the stress is at a plateau before the starting of the densification.

As it can be appreciated by the sequence of images in Figure 5b, stretching-dominated structures deform along a 45° orientation at the plateau stress, before undergoing densification. Instead, bending-dominated structures deform gradually but consistently over the entire cross-sections of the sample.

(a) Stretch-dominated structure Bending-dominated structure

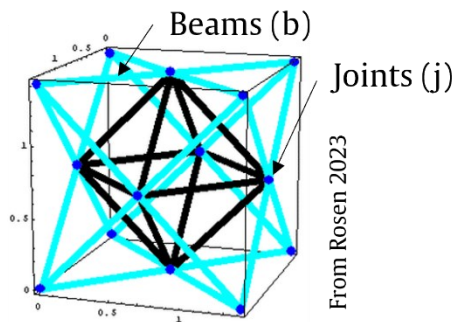


From Zheng X., 2014



From Benedetti M., 2021

(c) Octet lattice unit cell



From Rosen 2023

$$b = 36 \text{ (number of beams)}$$

$$j = 14 \text{ (number of joints)}$$

$$M = b - 3j + 6 = 36 - 42 + 6 = 0$$

↓
Stretch-dominated structure

Figure 5 a) Mechanical behaviour of lattice structures depending on whether they are stretch-dominated (on the top left) or bending-dominated structures (on the top right); b) Stress-strain curves relative to the compression behaviour of stretching-dominated (on the left) and bending-dominated lattice structures (on the right). Below, the frames with the three consequential stages that occur in the lattice compression; 1) linear elastic region; 2) plateau stress region; and 3) densification region; c) demonstration of the stretch-dominated behaviour of the octet lattice with Maxwell's stability criterium. Images adapted from Zheng et al. [91], Benedetti et al. [86], Rosen et al. 2023 [92].

In principle, it is possible to predict which type of mechanical behaviour will dominate by the lattice topology.

Maxwell's stability criterion (Equation 3) gives a first indication of the mechanical behaviour of a specific lattice structure based on the number of struts (b) and joints (j) in the unit cell:

Equation 3

$$M = b - 3j + 6$$

If $M = 0$, the structure is statically and kinematically determined: it is rigid and does not fold up when loaded, and its struts would be axially loaded, therefore a lattice with $M=0$ is classed as stretching-dominated [80, 87]. If $M>0$, the structure is over-constrained and would exhibit a stretch-dominated mechanical response when a load is applied [70, 87]. On the contrary, when $M<0$, the structure becomes a mechanism and has at least one degree of freedom, resulting in a bending-dominated response to external loads [87].

As the octet lattice has 36 struts and 14 joints, its $M=0$ and therefore it is a stretch-dominated structure (the reader is referred to Figure 5c for the demonstration). Nonetheless, L-PBF-manufactured octet lattices often behave as bending-dominated structures [70, 72, 86].

It is worth explaining that the Maxwell's criterium is accurate under the conditions that 1) the struts are sufficiently slender, i.e. that the relative density is sufficiently low (typically lower than 0.3) [86, 88]; 2) the struts are ideally aligned within the unit cell (the axes are aligned with the designed struts' orientation); 3) the struts exhibit homogeneous thickness.

Whilst condition 1 may be achieved by selecting the adequate unit cell design and suitable L-PBF process parameters, conditions 2 and 3 are challenging to obtain by L-PBF. Indeed, the authors [10, 72, 86] explain the experimental bending-dominated behaviour of L-PBF octet lattices by the misalignment and waviness of the struts in the fabricated lattices, which would introduce spurious bending moments to the beams [86].

As the accurate prediction of the lattice stiffness and mechanical behaviour becomes particularly challenging for additively manufactured structures [85], it is preferable to demonstrate the experimental mechanical behaviour of the L-PBF fabricated lattice structures. This is particularly relevant for zirconium lattices, for which no published work is available, and even the bulk material manufactured by AM has been little investigated. The standard that regulates the mechanical assessment of lattice structures is the ISO 13314 [93], which is relevant to porous metallic structures which exhibit a porosity of at least 50% (i.e. a relative density of 50% at most). This norm [93] is widely employed in the testing of cellular structures aimed at orthopaedic implants [10, 15, 67, 80, 86, 94, 95]. The ISO 13314 [93] enables the assessment of the stiffness – which is of particular interest in orthopaedic applications - and other mechanical properties relative to cellular materials. Indeed, the ISO 13314 tests are typically used to characterize the “elastic gradient”, that is the elastic modulus of lattice structures.

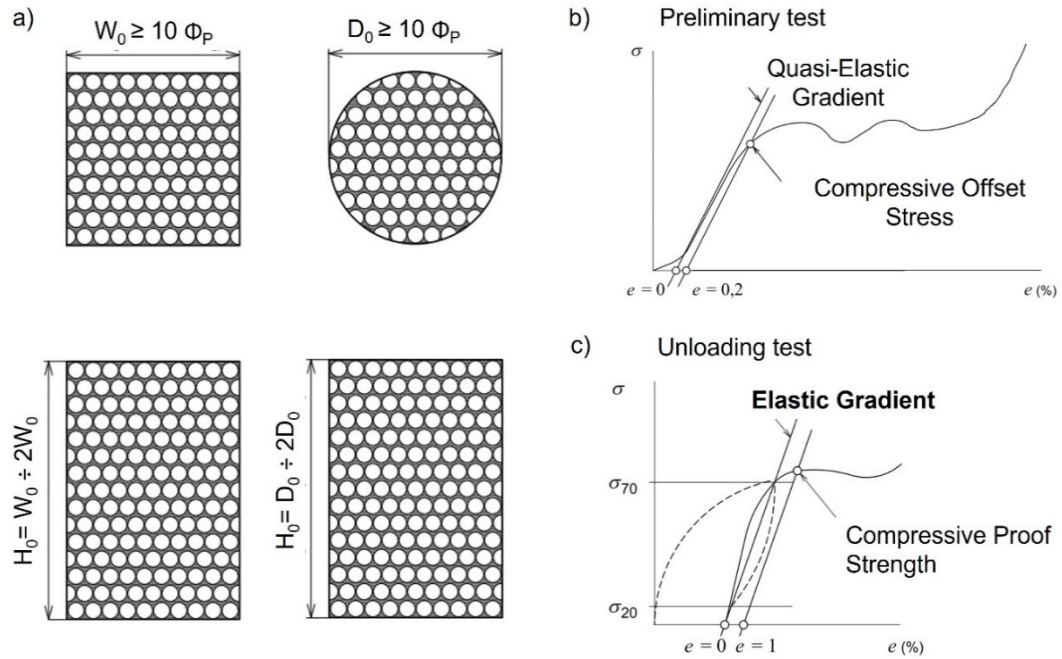


Figure 6 Schematic illustration of prescription of ISO 13314 on the sample geometries (a), preliminary compression test (b), and unloading test (1c) for porous materials; a) highlights the minimum width (W_0) or diameter (D_0) in relationship with the average pore size (Φ_P), and height constraints (H_0); b) shows a representative stress-strain curve for a preliminary test and the relative quasi-elastic gradient; c) is a representative stress-strain curve for the unloading test, where the elastic gradient is highlighted. The elastic gradient is the stiffness of the structure.

It is worth clarifying how the elastic gradient is determined according to the norm and explaining the principles of the mechanical tests that are performed to characterise this critical mechanical parameter.

In order to characterise the elastic gradient, the lattice structures (sample geometry illustrated in Figure 6a) need to be tested under two separated tests, which are shown in Figure 6b-c. The first, referred to as “preliminary test” consists of a quasi-static compression test aimed at evaluating the plateau stress (the second stage of the lattice and cellular structures), as illustrated in Figure 6b. As the name suggests, this test is preparatory for the second one, and it determines the loading conditions of the “unloading test” (that is the second test, Figure 6c). In the “unloading test”, a pristine lattice specimen is loaded between the 20% and the 70% of the plateau stress to close a hysteresis loop (see Figure 6c). The slope of the secant line obtained from the hysteresis loop is defined as the elastic gradient of the cellular structure [93]. The slope of the linear elastic region derived by the stress-strain curve from the preliminary test (Figure 6b) is defined as “quasi-elastic gradient” and should be used exclusively to determine the zero point of the strain. Nevertheless, the quasi-elastic gradient is often used in the literature to characterise the stiffness of metallic lattices and foams [86].

2.5.3 The lattice dimensions: a trade-off between functionality and manufacturability

The aim of this section is to highlight the rationale behind the choice of the dimensions for the key elements of a lattice structure: strut size, architectural pore size and architectural porosity.

The considerations illustrated here were derived by reviewing the literature available on the metallic lattices for implants, made in Ti6Al4V, Tantalum, etc. The principles described are valid for lattice structures to be embedded into any metallic implant, and therefore are applicable to the commercially pure Zirconium Zr702 used in this study.

The architecture of lattices for orthopaedic implants affects the interaction between the implant and bone tissue and contributes to the achievement of osseointegration.

Architectural porosity and architectural pore size are directly connected to the biological performance of the implant, influencing the recruitment, adhesion and proliferation of bone cells, but they also strongly affect the mechanical properties of the prosthesis [12]. For example, Yuan *et al.* [83] highlight the need for well interconnected architectural (open) porosity and suggest that the minimum architectural pore size is 100 μm , although pores in the range of 200-350 μm would support the bone ingrowth. Balla *et al.* [96] believe high architectural porosities are desirable. However, in their study, it was shown that after 11 days of culture on Tantalum porous samples, there was no significant difference in the biological response when comparing samples with different porosity (27% vs 45%). Liang *et al.* [97] suggested that osteoblasts⁶ might be able to bridge (and therefore to spread and migrate) only on pores smaller than 200 μm , although they acknowledged that higher porosities (hence greater pores) might promote their differentiation and, hence, the mineralization process (bone formation).

At the same time, the architectural pore size as well as the strut size are limited by the technical constraints of the technology used: PBF systems enable to melt specific areas of the powder bed with a limited resolution, which depends on the specific machine used and affects the minimum welding track achievable. Moreover, the lattice topology is constrained by the critical building angle: the minimum angle between the building platform and a beam/wall to be (successfully) manufactured by L-PBF without additional supporting structures. The critical building angle is typically 45° [98].

As a result, the lattice design for metallic structures that are to be fabricated by L-PBF is the result of a trade-off between functional (bio-mechanical) needs and (manufacturing) technological limits. From a bio-mechanical perspective, the key factors that contribute to the success of a lattice structure in an orthopaedic application are the extent of architectural porosity, the pore size, and the resulting compressive elastic gradient (which

⁶ Osteoblasts are staminal cells and the precursors of bone cells. Their differentiation leads to the so-called “mineralization process”, that allows the formation of the mature bone.

depends on all the lattice geometrical features: topology, architectural porosity, etc.) and general mechanical behaviour. The level of each feature depends on the specific lattice topology used and on the anatomical bone that has to be substituted, although in general it is recommended to have an architectural porosity (P_A) of at least 50% [10, 72, 77], with architectural pores ranging between 100-800 μm [10, 77].

From a manufacturing perspective, lattice geometry is constrained in terms of minimum strut size, pore size, and orientation of lattice surfaces and strongly depends on the L-PBF machine.

A scheme of how lattice design features are affected by the functional (bio-mechanical) requirements and manufacturing limits of L-PBF can be found in Figure 7.

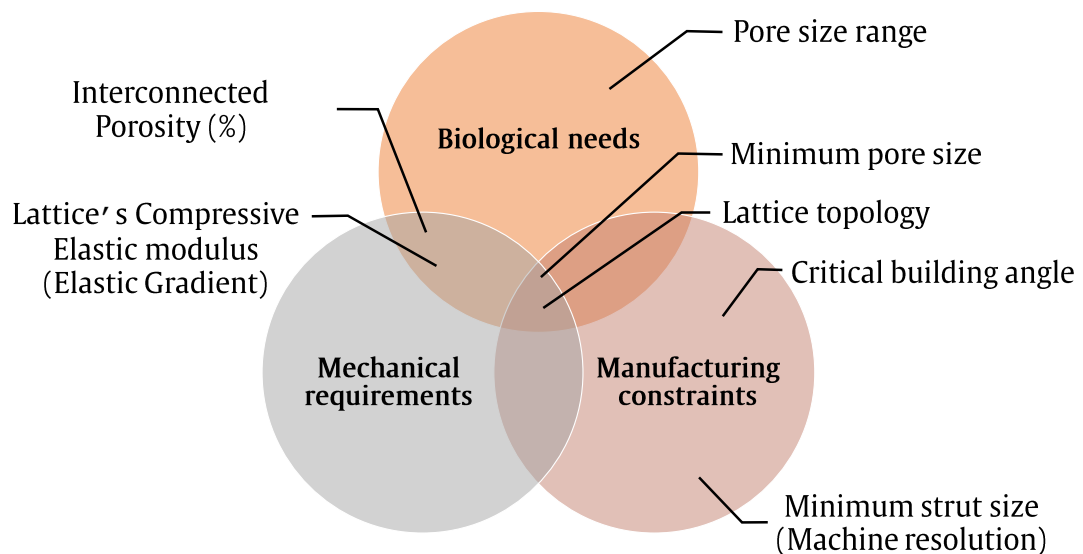


Figure 7 Design criteria for lattice structure for orthopaedic application should be based on the biological and mechanical requirements and the technological constraints in order to design functional and feasible lattices. Notice how several architectural lattice features (lattice topology, pore size, interconnected porosity, and lattice elastic gradient) need to be chosen based on a trade-off to meet different requirements.

In order to choose the lattice parameters (unit cell, pore size and porosity, and strut thickness) for this study, the design space for octet lattice proposed by [10, 72] and reported in Figure 8 was exploited. The grey area in this diagram is the result of a careful evaluation of L-PBF manufacturing capabilities and bio-mechanical requirements.

This graph is built onto the pore size axis and the strut size axis, and the design space was defined by curves characterised by the same unit cell size (Iso Cell-Size) and same architectural porosity (Iso Porosity). The minimum strut size is 0.2 mm, as per L-PBF manufacturing limits, the minimum architectural porosity is set to 50% for bone ingrowth, and the maximum pore size is set to 0.65 mm.

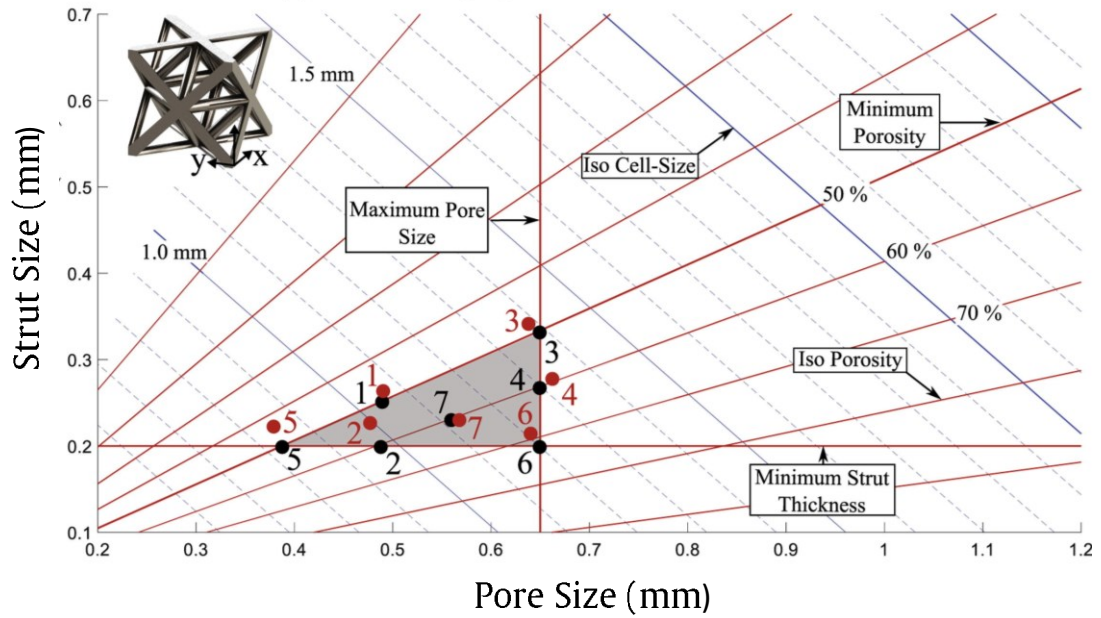


Figure 8 Design space for octet lattice proposed by Melancon et al. [10]; strut size is constrained by manufacturing technology employed (L-PBF), whereas pore size and porosity limited were chosen following bone ingrowth requirements found in literature. The grey area defines the design space for octet lattice for bone substitution. Black points refer to the as-designed structures, whereas red points refer to the as-manufactured structures obtained in the experimental study by Melancon et al. [10].

The so-defined design space is rather small, considering that strut size may be varied between ~0.2-0.35 mm, with unit cell dimensions ranging from ~0.8 mm to 1.4 mm and architectural porosities in the range of 50%-71%.

2.6 The challenges in the fabrication of lattices with L-PBF and the need for process optimization

As discussed above (§2.5), lattice structures for orthopaedic implants should exhibit an architectural porosity (P_A) of at least 50% [10, 72, 77], with architectural pores ranging between 100-650 μm [10, 77], regardless of the specific lattice topology. In addition to

this, the lattices should support the applied loads and be characterized by a compressive elastic modulus as close as possible to the substituting bone [8].

Hence, it is critical that the manufactured lattices are compliant with the as-designed geometry to ensure that the implants perform as expected.

Unfortunately, orthopaedic-relevant lattices have feature sizes close to the L-PBF manufacturing accuracy limits. Moreover, the quality of the lattice struts and nodes that is achieved by L-PBF is strongly affected by their orientation with respect to the building platform (for example, 45° inclined struts exhibit the staircase effect, whilst vertical struts display the pile-dish effect) [80]. Accordingly, the fabrication of these structures *via* L-PBF is challenging [10].

Depending on the process settings, the strut thickness might be higher or lower than designed (affecting the pore size and the architectural porosity), occluded architectural pores may be generated, and nodes may subside due to “sagging” [99]. Overall, these features affect the *geometrical accuracy* of the fabricated lattice.

Poor control of L-PBF parameters might lead to broken struts and through-holes in the nodes, which would disrupt the lattice connectivity between struts and nodes, that the author defined here as “*macro-structural integrity*”.

Beside the geometric compliance and macro-structural integrity issues, L-PBF-fabricated implants may exhibit internal voids or cracks, which compromise the *micro-structural integrity*. These defects decrease the bearing area and act as a stress concentration factor in the structure [100]. Hence, they could lead to a premature mechanical failure of the component. As such, the amount of defect porosity in the lattices must be limited.

To minimise these issues, the L-PBF process needs to be optimized for the specific lattice structure and material.

The current knowledge and understanding of L-PBF is still preventing the selection of suitable L-PBF process parameters for the fabrication of lattice structures *a priori* (that is,

with predictive models⁷), and the L-PBF optimisation normally goes through experimental trial and error approaches [2, 99, 101, 102].

In the case of L-PBF processing of Zr702, the (public) knowledge is very limited, with the work of Aristizabal *et al.* [7] published in 2019 representing the foundation study for the manufacturing of this material with L-PBF. Their study [7] allowed to identify optimal L-PBF settings for printing of bulk (fully dense) components. These were a laser power (P) of 250 W, a scanning speed (v_s) of 1250 mm/s, and a hatch spacing⁸ (h_s) of 75 μm . However, the review of the literature on the L-PBF manufacturing of other materials [2, 10, 72, 78, 99, 102] suggests that the optimal parameters found in [7] would likely be unsuitable for fabricating orthopaedic-relevant lattice structures. This hypothesis is based on two considerations. First, the L-PBF settings identified by Aristizabal *et al.* [7] were optimised with the sole objective of minimising the process-related porosity (P_p) in a full cube-like sample, whilst L-PBF lattices for orthopaedic application should satisfy additional constraints (the geometrical accuracy, for example). Second, even if the optimisation targeted the sole micro-structural integrity objective (minimum P_p) of the lattice structure, the application of the same bulk-optimal settings as [7] would not ensure similar outcomes in terms of densification/micro-structural integrity, given the different heat transfer and solidification mechanisms that are involved in the generation of bulk and lattice structures [2, 103, 104].

At the current stage, there is no clear understanding on how the process parameters (such as the laser power or the scanning speed) should be varied when moving from the bulk L-PBF optimal parameters to the lattice L-PBF parameters. The issue is exacerbated by the limited literature available on the role played by specific L-PBF process parameters on the

⁷ It is worth noting that increasing effort is put in generating predictive models for the optimisation of L-PBF for the manufacturing of bulk components. The most important work in this sense is represented by the study of Bajaj *et al.* (*Predictive process parameter selection for Selective Laser Melting Manufacturing: Applications to high thermal conductivity alloys*, Additive Manufacturing, 2019, 27: p. 246-258), who proposed a heuristic based on normalised process maps and analytical predictive models for heat transfer and melt pool size.

⁸ The hatch spacing (h_s) is the distance between the midlines of two adjacent molten track in the powder bed, as illustrated in Figure 10.c.

lattice geometrical accuracy, micro-structural integrity, and macro-structural integrity. For example, a few studies have focused on the influence of the L-PBF process parameters on the geometrical accuracy of lattices, which are notably performed by the research group led by Attallah at the University of Birmingham [2, 99, 102] and by Pasini at McGill University in Montreal [10, 72, 77, 78, 105], whilst the materials investigated were Ti6Al4V, TiNi, and AlSi10Mg. Fewer L-PBF optimisation studies focusing on the role played by L-PBF parameters on the lattice micro-structural integrity were found [2, 102], and no research has yet focused on the generation of occluded pores or on the lattice macro-structural integrity.

The lack of knowledge on the processing of Zr702 for the manufacturing of lattice structures highlights the need for optimisation studies that would allow 1) to improve the understanding of the influence of single process parameters on the various lattice features (strut size, architectural porosity, process- related porosity, etc.), and 2) to identify suitable process parameters for the printing of lattice structures for zirconium orthopaedic implants.

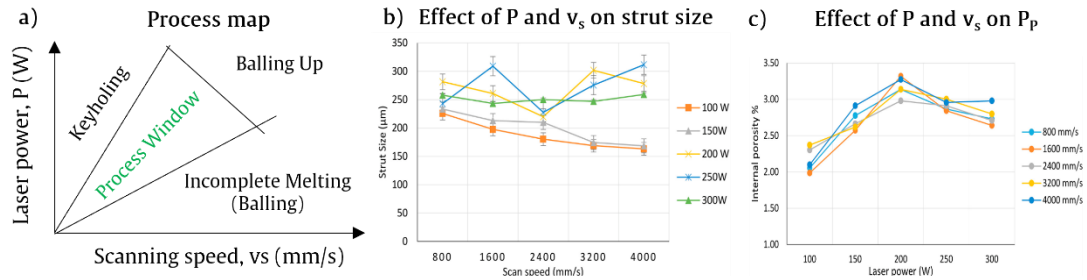


Figure 9 a) Process map for L-PBF manufacturing, in which the process window with the optimal levels of P and v_s can be rapidly identified; b) Experimental results by Salem et al. [99] showing the interaction between P and v_s for the strut size; c) experimental results by Salem et al. [99] showing the interaction between P and v_s for the process-related porosity (P_p).

The main parameters that need to be optimised are the laser power P and the scanning speed v_s , two parameters that are often used to generate “process maps” in which the “process window” can be rapidly identified (Figure 9a). The influence of P and v_s on the strut size and the process-related porosity is rather complex: the effect of one parameter often depends on the other one (i.e. interaction between P and v_s exists), as clearly

demonstrated by Salem *et al.* [99] (who worked on Ti6Al4V lattices) and graphically shown in Figure 9b-c.

Additional settings that depend on the scanning strategy (i.e. the specific path followed by the laser to melt the desired regions of the powder bed) should be optimised.

The L-PBF optimisation of Zr702 octet lattices with the full range of scanning strategies is beyond the scope of this thesis. However, the optimisation of the L-PBF settings will be focused on two widely employed scanning strategies, namely the “island strategy” and the “contour strategy”, which are schematically shown in Figure 10.

The first is arguably the most frequently adopted scanning strategy, as it is often employed in the manufacturing of fully dense objects. Notably, it was used to generate of Zr702 bulk disks in the work of Aristizabal *et al.* [7] is recommended to minimise the residual stresses in the L-PBF-fabricated components [106, 107]. With this printing strategy, each CAD layer that composes the designed object is divided into small squares (called “islands”, typically ranging between 2-7 mm [1]). In the L-PBF machine, the laser scans each island individually with a meandering pattern, filling. The orientation of the scanned tracks of a single island results perpendicular to its directly connected neighbours, so that the solidified layer appears like a chessboard (see Figure 10.a).

The hatch spacing (h_s) - that is the distance between two adjacent scanned tracks, as shown in Figure 10.c - represents an important L-PBF parameter in the island strategy. The h_s determines the overlap between the molten-solidified material and affects the energy that is inputted onto the powder bed during the manufacturing process through the areal energy density (E_d , J/mm²), which is defined in Equation 4.

Equation 4

$$E_d = \frac{P}{v_s \times h_s}$$

The author acknowledges that the energy density that is often referred to in the literature [106, 108] is the *volumetric* energy density and expressed in J/mm³. The so-defined parameter is simply the ratio between the areal energy density (defined in Equation 4)

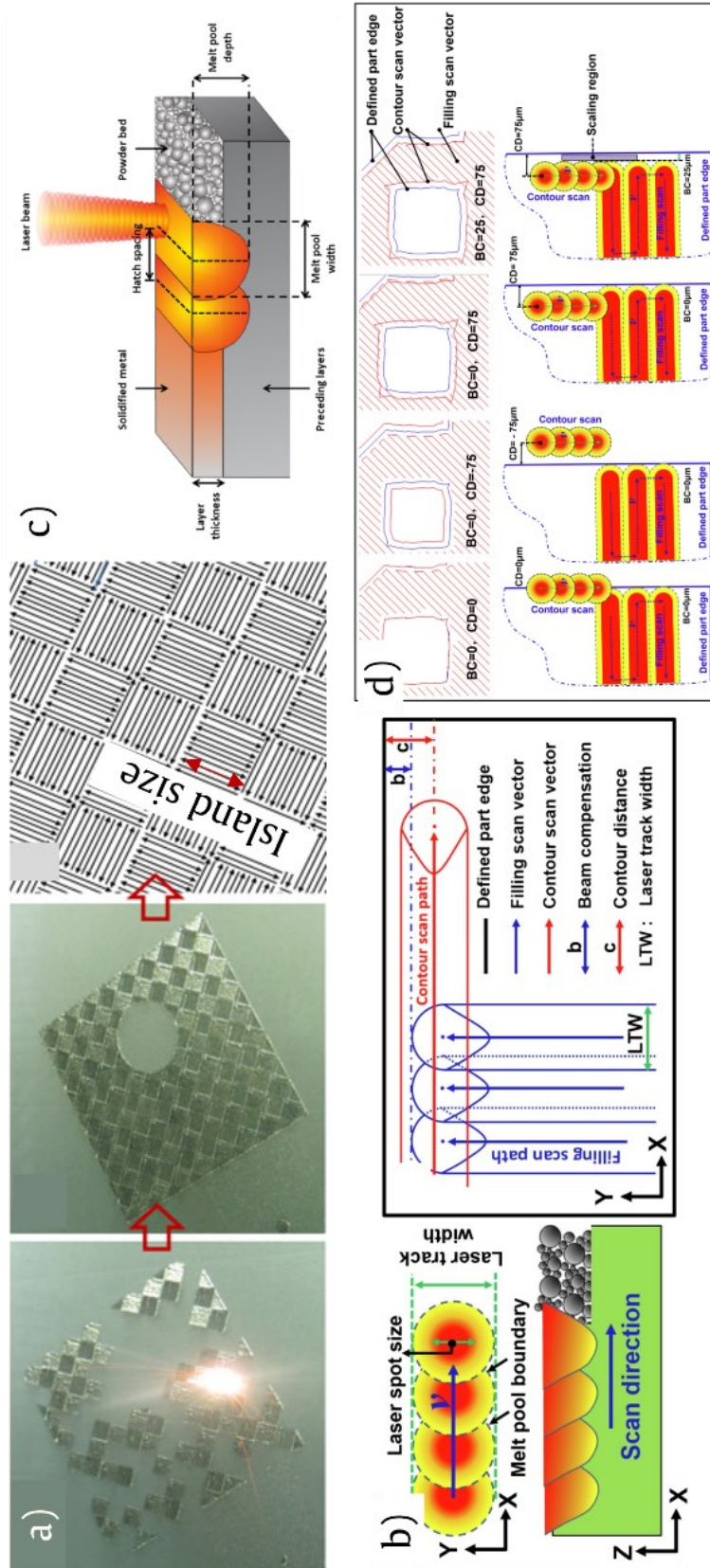


Figure 10 Schematic illustration of: a) Island strategy, in which the powder bed is progressively invested by the laser beam, into small squares (islands); b) Contour strategy, in which the edges of the components are molten with a contour scan track; c) Hatch spacing; d) Contour distance and beam compensation parameters. Images adapted from [1-3].

and the thickness of the layer deposited onto the bed. However, the author prefers to use the areal E_d because the thickness layer is imposed by the powder size, rather than constituting an adjustable factor in process optimisation studies. It is also worth mentioning here that several authors discourage the use of the volumetric E_d to guide the L-PBF process optimisation, as it fails to predict accurately the quality of the manufacturing [109].

As it affects the E_d , the hatch spacing needs to be adjusted to ensure that the energy density is suitable to melt the full layer of powder bed as well as to weld the molten tracks to the solidified material beneath, whilst avoiding excessive heating which would lead to keyholing. The h_s affects the P_p , mechanical properties, residual stresses and surface finish of the fabricated component [110]. However, it is not clear if it has a determining role in the geometrical accuracy of L-PBF manufactured objects.

The contour strategy – schematically illustrated in Figure 10.b – is characterised by the contour track, which runs along the edges of the objects at each layer during the L-PBF manufacturing. The internal areas of the objects are typically molten with a meander pattern or filling. The contour strategy is recommended to improve the geometrical accuracy and micro-structural integrity of thin walls and lattice structures in general [2, 111, 112].

In this case, two parameters, namely the laser beam compensation (BC) and the contour distance (CD) are normally adjusted with the intent of reducing the geometrical mismatch between the designed and the manufactured component [2]. The BC is the distance between the filling/meander scan vector and the ideal component edge, and therefore acts on the filling of the internal areas of the component, as illustrated in Figure 10.b and Figure 10.d. The CD, instead, is the distance between the midline of the contour scan track and the edge of the designed part (see Figure 10.b and Figure 10.d).

2.7 The characterisation of L-PBF lattices with X-rays Computed Tomography

As previously illustrated, the manufacturing of lattices by L-PBF is challenging and the fabricated structures are typically affected by some geometrical mismatch with the designed lattice, undesired internal pores, and might exhibit poor structural connectivity. However, assessing these features (determining the geometrical accuracy, the micro and the macro-structural integrity) in L-PBF lattices poses several challenges. First, these structures are constituted by intricate thin struts that are difficult to access and measure, let alone to assess in terms of structural connectivity. Second, internal pores are inherently inaccessible and their assessment on 2D microscopies made on a few cut sections (hence, by destructive testing) on the manufactured lattice may not be representative of the overall P_p .

X-rays Computed Tomography (X-CT) is a non-destructive testing (NDT) that allows the scanning of objects made by relatively low-density materials to acquire and re-construct their volume in 3D.

The working principle is the following: an X-rays beam is directed onto the sample of interest, and the transmitted X-rays are collected by a detector. Based on the reduction of the X-rays intensity, X-rays absorption images are generated and subsequently subjected to mathematical operations to reconstruct a volumetric data set. The single unit of information of the data set is called a “voxel” (from “volumetric pixel”) and contains a brightness level that is associated with the absorption of the radiation from the material during the scanning. This information allows for the estimation of the density of the material that is represented by each voxel, enabling the identification of different phases of the scanned object across the volume, as well as the detection of internal and external edges.

X-CT offers the unique capability of simultaneously acquiring the data needed to evaluate the geometric accuracy, material integrity and surface quality of a part. Importantly, it

enables the volume reconstruction of unique and complex geometries (including re-entrant features, free-form surfaces and lattice structures) and is applicable to a variety of materials (titanium, aluminum, etc.). Process-related porosities P_p generated during the manufacturing can be identified, isolated, localized and measured through the whole L-PBF component, overcoming the limits of 2D microscopy. Furthermore, the structural connectivity of lattices can be assessed visually through the whole sample.

In summary, X-CT represents the perfect tool to characterize the above-mentioned lattice features and to gather useful information from the fabricated structure to guide the optimization of the manufacturing process.

The technique is interesting also from an industrial perspective, especially for the biomedical sector. It is relatively easy and “clean” as no sample preparation is needed. As such, there is no risk of leaving residual substances onto the material surfaces, which is a great advantage for medical industry. Moreover, it allows for the assessment of single-unit products as well as mass-produced items in a non-destructive way, which offers the opportunity to control the quality of implants without sacrificing them and to support the economic sustainability of mass customization.

X-CT has attracted the interest of many as a NDT technique for the characterization of L-PBF lattices, implants and other components [71, 113-115].

Du Plessis *et al.* [113] proposed X-CT as a quality control technique for L-PBF full density (bulky) medical implants and demonstrated its suitability for the detection of microporosities and internal cracks which might occur in the fabrication due to poor manufacturing process control. Moreover, they proposed a straight-forward method to evaluate the dimensional accuracy of a nasal cavity implant, which was assessed by comparing the surfaces of the reconstructed volume with the CAD file and calculating the distance (variance) between the surface elements of the two.

The same approach was used later by Du Plessis *et al.* [71] to determine the geometrical mismatch of diagonal and rhombic lattices. However, these methods are only available in

special software, such as Volume Graphics VGStudioMax, and do not allow to precisely characterize the lattice geometry.

Lozanovski *et al.* [116] characterized the geometrical accuracy of lattice nodes of a faced-centered cubic lattice with an automatic image analysis. However, their protocol enables the sole characterization of nodes and would fail when used on lattices with horizontal struts, like the octet lattice.

Bagheri *et al.* [78] characterized the lattice strut size, the architectural pore size, and the architectural porosity. Nevertheless, the author noted that the protocol used for geometrical characterization of struts and pores is not detailed and seems to be based on manual measurements.

Interestingly, no work was found on the characterization of the macro-structural integrity of lattices, although the connectivity of the struts and nodes in the structure strongly impacts the mechanical performance of the lattice structures, and the 3D reconstruction obtained by X-CT would allow evaluations in such terms.

In conclusion, the X-CT has been successfully used to assess the micro-structural integrity of lattices and some attempts to characterise the geometrical accuracy have been made. At the current stage, however, only the geometry of lattices made by struts exhibiting only one orientation with respect to the building platform (for example, 45° from the building platform like FCC, rhombic or diagonal lattices) have been assessed. Moreover, the dimensional characterisation of lattices was often performed manually (hence with lengthy operations) or by using specialistic software (with the related costs). The lack of a tool that allows the prompt assessment of the geometrical accuracy for lattices with struts in multiple orientations, such as the octet lattice, motivates the development of an automatic image-based tool that can characterise both nodes and struts of octet lattices.

2.8 Challenges and opportunities in the measurement and characterisation of surface topography of L-PBF lattices for the orthopaedic application

This section aims at illustrating the challenges and potential solutions for the topographical characterisation of orthopaedic-aimed L-PBF lattice structures.

First, the role of the surface topography in the osteointegration of orthopaedic implants is discussed, and then the influence of manufacturing processes on the surface topography is illustrated. As it will be demonstrated later, surface topography acts as a bridge between the manufacturing process and the manufactured component's performance.

The typical topographical features of L-PBF surfaces are presented, and an extensive discussion is devoted to a particular L-PBF feature: the partially melted particles (PMPs). Finally, the available methods for the detection of PMPs will be presented and discussed, highlighting the gaps that need to be overcome for a topographical characterisation of L-PBF lattice structures. Furthermore, the potential of using a "PMPs density" parameter for a synthetic surface description will be discussed.

2.8.1 Surface topography: a bridge between the manufacturing and the implant performance... which is still to be built

Surface topography is defined as "the entire geometric information associated with a surface shape and its features" [117]. The geometrical irregularities of the surface excluding those contributing to the form are referred to as surface texture [117].

Topography plays an important role in determining several performances as well as the service life of the whole component [119] (notably when this is made by AM [118]). Indeed, topography affects the components' corrosion resistance, tribological properties and fatigue life [122].

In the case of biomedical implants, topography plays an additional and crucial role, since it affects protein adsorption and cell adhesion onto the implant, and, therefore, the prosthesis osteointegration and implantation outcome [11].

Submicron-topography seems to play a crucial role in protein adsorption, one of the firsts events after implantation, while micro-topography is believed to have a major effect on cell adhesion and proliferation [123]. For example, it has been suggested that topographical cues may guide the cell spreading onto a surface [124] by “contact guidance” [125], and it has been demonstrated that cells are sensitive to curvature variations of cellular scaffolds [126].

With respect to prosthetic devices for bone replacement, it has been shown that rougher surfaces tend to be colonized by osteoblasts (bone cells) in shorter times, potentially leading to faster and better osteointegration [124] and shorter healing period for the patient [123, 127].

However, although extensive research is being carried out in this area [125, 126], the relationship between biomaterial topography and cellular adhesion is still not clear, and available data are often contradictory [127]. For example, there is no agreement in the literature on the most favourable texture for an earlier cell colonization [123].

If it's true that biological studies are prone to experimental variability and that the subject is inherently complex (cellular behaviour is not just affected by topographical cues but by chemical signals as well [125, 128, 129]), the author believes these inherent challenges are exacerbated by a poor characterisation of biological surfaces. By reviewing the literature on the surface topography-cellular response relationship [125, 126, 128, 130-138], it was found that the surface characterisation is still widely based on profilometric data (2D data), topographical filtering and data treatment are not completely detailed/disclosed, and even surfaces that exhibit special topographical features or patterns are often described solely through roughness parameters like the average roughness (Ra), the root-mean-square-roughness (Rq), or their areal counterparts (Sa and Sq). Unfortunately, such topographical parameters – whilst still potentially relevant to the surface function – are not able to fully describe the surface texture and, indeed, very different surface topographies might still exhibit the same summary indicators.

Perhaps, then, a different strategy in the characterisation of biological surfaces is needed if we want to shed a light on this subject and finally understand what makes a “good” surface for osteointegration.

Replying to this open question might also lead to establish guidelines for the manufacturing and post-processing of osteointegration-aimed devices: whilst having a strong impact on several component’s properties, topography is, in turn, highly affected by the manufacturing process used in the production of the components. In particular, surfaces are made recognizable by the industrial process so that the signs left by the manufacturing operations are often referred to as the “process signature” [139] [140] or “fingerprint” [141]. Because topography is affected by manufacturing process parameters, surface topography becomes a quality index of the manufacturing operation. Therefore, topographical investigation may be a valuable tool to infer and reconstruct the physical phenomena that take place during the process [139].

As demonstrated above, topography bridges the manufacturing process with the performances of the manufactured component [142]. In this sense, the investigation of topography of manufactured objects may resembles to “scientific chiromancy”, where lines and undulations are used to infer part of the surface history and to estimate the component’s performances.

However, this bridge (or chiromancy) is still very much to be engineered and built, both from the side of the L-PBF manufacturing process and from the side of “osseointegrative” function. The tool that enables to bridge these gaps is surface metrology, which is the science that measures and characterizes the surface topography and allows for such type of investigation [117].

2.8.2 L-PBF-generated surfaces and lattice structures and the issue of their characterisation

The surfaces generated by L-PBF are generally described as “irregular”[117, 138], “complex” [143], or “rough” [122].

The texture varies depending on the orientation of the surface with respect to the building direction, the quality of the powder used in the manufacturing, the printing configuration (e.g. contour strategy, pulsed or continuous laser power, etc) and, in general, the interaction of the material with the laser [144-147].

Normally, upward flat surfaces exhibit weld tracks, weld ripples, and globular structures, which are normally due to the solidification of material ejected from the powder bed during the manufacturing, typically referred to as “spatter”. Side, inclined and down-ward surfaces, instead, are characterised by the so-called “partially melted particles” (PMPs), which are spherical-like particles that may be found in single or cluster configuration. Furthermore, vertical and inclined surfaces are characterised by the “stair-case effect” or “dish-pile effect”.

L-PBF lattice structures consist of struts that are created by melting material along different directions on top of the building platform and, therefore, exhibit all the above topographical details.

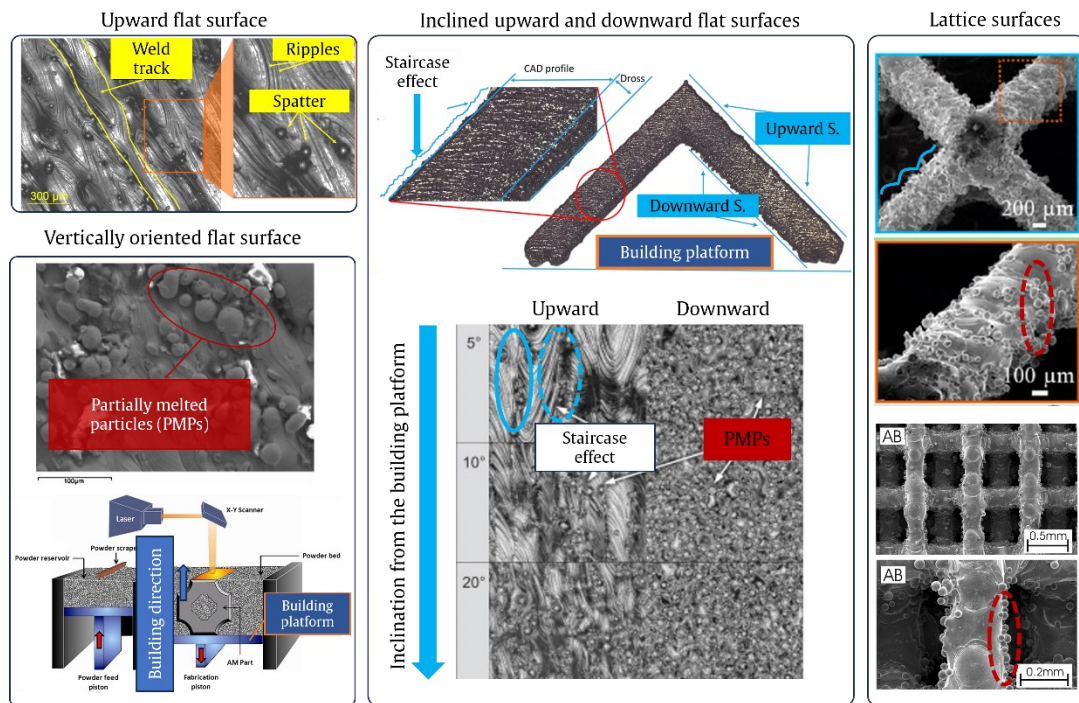


Figure 11 The quality of L-PBF surfaces created at different orientations with respect to the building platform, and the aspect of L-PBF lattice structures in their as-built (AB) condition. Images were adapted from [3, 122, 139, 148-150]

With such characteristics, areal topography measurement is necessary to capture the details of surfaces generated by L-PBF. However, if the actual measurement of L-PBF-generated surfaces and L-PBF lattice is already challenging due to surface reflectivity and accessibility (just to cite a few), the characterisation of such surfaces is also problematic. Traditionally, surface texture is decomposed into “roughness” and “waviness” and areal texture parameters are measured following the ISO 25178-2 in the attempt to describe an entire surface by summary indicators (relative to either the waviness or the roughness). Whilst being very popular, however, the characterisation based on texture parameters poses two problems. On one hand, it fails to provide insightful information on the typical features and marks left by the manufacturing process [139]. In this regard, Senin *et al.* [139] reported a “missed opportunity” in the full exploitation of the topographical data acquired over L-PBF-manufactured surfaces when only texture parameters are measured. On the other hand, the calculation of such summary indicators is hardly possible onto complex-shaped surfaces, such as those of lattice structures, due to the clear challenges in the separation of roughness from waviness [144]. In fact, Thompson *et al.* [151] wonder if conventional filtering methods and texture parameters are appropriate at all on additively manufactured surfaces.

A shift in the surface characterisation is needed for L-PBF-manufactured surfaces (particularly for L-PBF lattices), that aims at complementing (or abandoning) texture parameters with (or for) feature parameters. Indeed, feature-based characterisation (FBC) is able to measure and analyse specific topographical formations. Some, such hills and dales, are already contemplated in the ISO 25178-2, but other features can be defined and introduced to describe special topographical details that pertain to L-PBF surfaces, like PMPs, weld tracks, or single melted layers. For this reason, FBC has been proposed for the analysis of such surfaces in the attempt to link the surface quality to the manufacturing process’ signature and the component’ s function [11, 139, 144, 152].

2.8.3 Partially melted particles (PMPs) and the potential risks for L-PBF lattice structures in orthopaedic implants

From a purely geometrical perspective, spatter and PMPs look alike (both are spherical-like particles that protrude from the surface), and their size is in the micro-scale, which is the relevant order of magnitude for (bone) cells and bacteria [11, 123, 153]. Therefore, the author assumes these L-PBF features would represent the same topographical cue for the cellular behaviour. As such, the term “PMPs” will be used hereafter to refer to both the spatter and PMPs, as it is often done [154].

As mentioned above, PMPs’ size is in the order of microns, and the size of single PMPs depends heavily on the source powder’s distribution and the manufacturing process settings [139].

Although some advocate for the direct use of L-PBF-generated surfaces in their as-built condition [134, 144], the presence of PMPs on surfaces that are aimed at permanent implants concerns many in the scientific community [69, 138, 155-157]. Xie *et al.* [156] demonstrated that PMPs are responsible for higher bacterial adhesion and inhibit the osteogenic activity in Ti6Al4V lattice structures. Dall’Ava *et al.* [12] expressed their concern over the presence of such features which were found in retrieved hip implants fabricated by AM, highlighting the risk of mechanical detachment or corrosion-driven removal of PMPs from the implant, potentially leading to PMPs entering the blood stream and cause osteolysis. Maszybrocka *et al.* [73] remarked that detached PMPs could also be transported to the articulation region of hip implants, where they would contribute to the “third-body wear” process. From an exquisitely mechanical perspective, PMPs and the irregularities of additively manufactured surfaces cause concerns over the fatigue strength of permanent prostheses [69].

Furthermore, the author would like to offer her perspective on the topic, that was inspired by the findings reported in study published on Nature by Pieuchot *et al.* [126], who demonstrated how cells migrate on cellular scaffolds towards concave valleys by a cellular mechanism called “curvotaxis”. As PMPs are surface protrusions which, however, do not

generate concave valleys, PMPs-bearing surfaces do not seem the ideal scaffold for bone tissue (re)generation.

Given all the above, it is unsurprising that there is great academic interest on surface engineering aimed at improving the quality as-manufactured lattice topographies by removing PMPs, with several researchers already active in this area [156, 158-162].

At the current time, however, there is no regulation that imposes specific standards of quality of on the topography of additively manufactured lattice structure (and porous features, in general): when they are embedded in commercial prostheses, lattice structures do not undergo any topographical modification and are not characterised. The result is that whilst we know that PMPs populate the surface of porous features in implanted prostheses, the amount (or superficial density) of PMPs and their characteristics (size, location, etc.) are unknown.

Nevertheless, the author believes the risks associated with PMPs should be taken in serious consideration among the regulatory bodies (i.e., FDA and EMA) and expects that standards on topographical quality of additively manufactured permanent implants will be developed in the future, particularly with respect to the amount and quality of PMPs. It becomes interesting, then, to develop methods that allows for the quantification and characterisation of PMPs.

2.8.4 Challenges and opportunities in the characterisation of Partially melted particles (PMPs) on L-PBF lattice structures

Acknowledging the challenges of characterizing lattice surface topographies (such as the difficulties in topography measurement and characterization, or the lack of standardised protocols, to mention a few), the author decided to focus on the development of a protocol to capture the PMPs on additively manufactured lattice structures and to propose quantitative parameters that would enable to characterise (and therefore compare) the surfaces topographies of lattice structures with respect to the PMPs.

Although some work has been done for the detection of the typical topographical features of additively manufactured surfaces including PMPs, no method developed so far has successfully been applied on lattice structures. Indeed, the topographical characterisation of additive manufactured surfaces in general is only at an infant stage [152], and most of the work is focused on flat horizontal surfaces, that is surfaces that are oriented perpendicularly to the building direction in the L-PBF.

Senin *et al.* [139] developed an algorithm to detect and characterise the “signature topographical features”, including the PMPs and the welding tracks over a flat L-PBF manufactured surface. Their method is based on *height thresholding* and involves image analysis and requires iterative operations to determine the “most stable cross-sectional contours over a vertical range (i.e., those that change less between selected threshold levels)” to isolate the PMPs over flat surfaces, as shown in Figure 12a. Unfortunately, this method is not suitable for the detection of PMPs over non-horizontal flat surfaces because the contours of PMPs would not be stable across the vertical direction.

Lou *et al.* [144] were able to isolate particle clusters on L-PBF flat surfaces with a *watershed segmentation method* (see Figure 12b), and highlighted the need to pre-process the topographical data by enhancing the edges of the particles and to post-process the segmented topography by Wolf pruning to avoid over-segmentation of the features. Whilst being a promising method, the robustness was not tested, and the author noted that protrusions that were smaller than 36 μm (indicated by red arrows in Figure 12b) were not detected, due to the pruning operation.

These methods were developed and calibrated for a specific case: upward L-PBF manufactured flat surfaces, characterised by weld tracks and PMPs. The same algorithms may be unsuitable on the lattice surfaces, which are characterised by an even more complex topography, as a result of the particular geometry.

To the best of the author’s knowledge, the only attempts of topographical characterisation of lattice or porous structures reported in literature are represented by

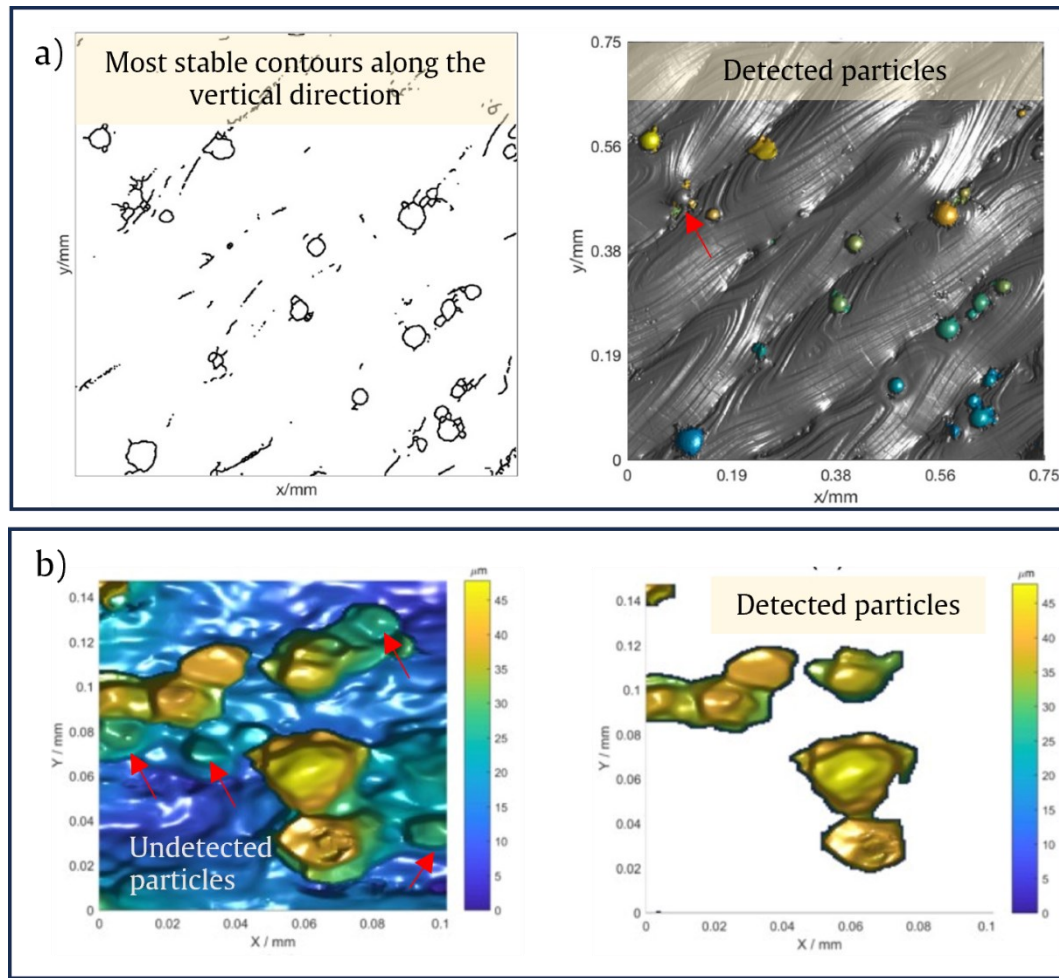


Figure 12 Illustration of successful detection methods for the topographical analysis of flat L-PBF generated surfaces. a) Detection method of Senin et al. [139]. On the left, the edge map which represents the intermediate segmentation step, in which the most stable contours (edges) of PMPs across the vertical direction can be found. On the right, the final segmentation of PMPs, which stand out clearly from the background. b) Detection method proposed by Lou et al. [144]. On the left, the colour map of the measured topography. On the right, the detected PMPs. The red arrows highlight the undetected particles on both the sample areas. Images adapted from Senin et al. [139] and Lou et al. [144].

the works of Maszybrocka et al. [73], published in 2017, Lou et al. [11], and Townsend et al. [163], both published in 2019. The three studies were performed on topographical data acquired by X-rays computed tomography (X-CT), a technology that allows to reconstruct the 3D geometry of complex objects (more details can be found in §2.7) and, therefore, potentially allows for the areal topography of internal and/or inaccessible features.

The first [73] characterised several amplitude parameters and the hybrid parameter “developed interfacial area ratio” (Sdr) and attempted a feature analysis to isolate the PMPs. However, the reproducibility of the work is impaired by the lack of a clear and

accurate procedure for the topographical analysis (the method of particle detection was not specified). Moreover, the feature-based characterisation was outlined on a single sample area, covering only a small area of a single strut. Nevertheless, the work has two merits: 1) performing topographical analysis on a high-resolution data set (voxel size = 1.9 μm , which is significantly smaller than single PMPs); and 2) demonstrating that common areal texture parameters are inadequate to describe and characterise the surface of L-PBF porous structures.

The second [11] characterised the porous features of an acetabular cup fabricated by Electron Beam Powder Bed Fusion, an AM technology that is similar to the L-PBF but uses metallic powder with larger particle size. In this case, the authors acknowledged the impossibility of calculating areal parameters according to ISO 25178-2, and exploited manifold areal parameters (S_a , S_q , S_{sk} , and S_{ku}) that were calculated based on a “computed reference form”. Furthermore, they used watershed segmentation and Wolf pruning (height pruning = 12 μm) to measure two feature-based parameters: the density of peaks and the density of pits. The authors suggested that these and other feature-based parameters like arithmetic mean peak curvature, mean daled area, etc.) might be able to link the surface quality of orthopaedic surfaces with the implant osteointegration. The characterisation method proposed there has great potential for building the bridge that connects the surface topography to the osteointegration function that was discussed in earlier sections. Nevertheless, the author believes that the values of the computed parameters presented in the study should be evaluated critically, considering that the resolution of the topographical data was 31.7 μm . Whilst the quality of metallic powder used to make the acetabular cup is unknown, the author has ample reason to suspect that the resolution is in the range of the particle size and the PMPs size. Indeed, the low resolution did not allow for the capture of PMPs, which were filtered out in the measurement, and the surface (mesh) appeared to be rather smooth. As a consequence,

the author suspects that the density of peaks greatly underestimate the actual concentration of hill-like features.

The third study [163] characterised PBF-generated lattices by an original Sdr parameter (“Sdr prime”), that is applied to the “true 3D data” from X-CT and that overcomes the challenges of ISO 25178-2 on AM surfaces. Whilst this new parameter is directly related to the specific surface of PBF-generated surfaces (including PBF lattices) and constitutes a significant progress in the lattice characterisation, Sdr is not capable of discriminating between concave valleys and convex features (like PMPs). As such, this method cannot fully describe the function of an orthopaedic-aimed PBF surface.

In conclusion, attempts to characterise PMPs on L-PBF-manufactured flat surfaces have been successfully made, showing the great potential of FBC on these surfaces. However, the methods developed so-far may not be suitable for the direct application to complex-shaped, almost free-form lattice surfaces. The development in this niche area is underway, with few works being published and many challenges to be addressed. At this stage, there is no (robust) algorithm that can detect and characterise the PMPs on lattice/porous structures. Finally, there seems to be an interest in exploiting X-CT to acquire topographical data of lattice surfaces, although the data resolution might be a limiting factor for the adoption of such techniques.

2.8.5 The density of PMPs for the characterisation of L-PBF lattice structures

Let us assume for a moment that we have at our disposal an algorithm that can detect PMPs onto L-PBF lattice structures. At this point, it might be of interest to describe the surface texture through a synthetic parameter, that would allow to rapidly compare different L-PBF lattices.

In the light of the considerations made above about the relationship manufacturing-PMPs-osteointegration, an ideal texture parameter would capture the severity of the PMPs adhesion onto the surface. Indeed, the amount of PMPs appears to be strongly affected by the particular process parameters used [164] and might also become a quality index for

orthopaedic (and osseointegration-aimed) surfaces manufactured by L-PBF. On the latter aspect, it is worth noting that surfaces with high peak density have been proven to inhibit cell proliferation [130, 165] and, therefore, it seems particularly appropriate to characterise the PMPs concentration on osseointegration-aimed L-PBF surfaces, as it provides functional characterisation of the topography.

The particle density, which is the ratio between the number of particles detected on a surface and the surface area, seems to be an ideal candidate for the purpose of synthetic texture description. The particle density is an appealing parameter because it is readily available and easy to interpret because it describes physical features that are immediately recognisable to manufacturers and biologists. Furthermore, the parameter's definition is not new, and therefore its use does not contribute to exacerbate the "parameters rush/rash". Indeed, some authors have already proposed it for the characterisation of L-PBF manufactured surfaces and other additively manufactures surfaces [73, 164].

In conclusion, the author believes that a "PMPs density" might be an ideal route for the topographical characterisation of these special surfaces, and that it can be easily adopted by both researchers in academia and manufacturers in industry, and that such parameter might be adopted across different sectors (biomedical, aerospace, automotive, etc.).

2.9 The knowledge gap and the objectives of this study

The literature review identified the following points and knowledge gaps.

Zirconium is seldom exploited or investigated in the biomedical field, albeit being biocompatible. The material was actually used in the '50s in England for orthopaedic implants. However, it was soon redirected to the nuclear sector and its biocompatibility was soon forgotten/neglected. Today, only a narrow community seems to be aware of its potential in orthopaedics (although its oxide, ZrO_2 , is widely exploited for the very same application). Aristizabal *et al.* [7] previously investigated Zr702 (commercially pure zirconium) in AM and confirmed the biocompatibility of L-PBF-fabricated bulk disks. However, the large stiffness mismatch between this metal and the bones represents a risk

of “stress shielding”. Lattice structures, which are characterised by a reduced stiffness compared to the material they are made of, constitute a potential solution to this issue. To the best of the author’s knowledge, the fabrication of (orthopaedically-relevant) zirconium lattice structures has not been attempted yet.

Fabricating lattices with good geometrical accuracy and structural integrity is critical to ensure the correct functioning of orthopaedic implants. It requires extensive L-PBF process knowledge, which is unfortunately lacking in the case of zirconium. Indeed, although Aristizabal *et al.* [7] identified a set of optimal L-PBF settings for the manufacturing of full disks, these conditions are likely to be unsuitable for the fabrication of orthopaedically-aimed lattice structures, given that: 1) the Aristizabal *et al.* [7]’s optimisation was aimed at minimising the internal porosity only (further constraints on architectural porosity and macro-structural integrity need to be considered for lattices); 2) the heat transfer and solidification mechanisms involved in the printing of bulk and lattice structures are inherently different.

As there is no public knowledge on zirconium lattice structures, their bio-mechanical behaviour is also not known.

With respect to their mechanical behaviour, the lattices’ stiffness depends on their topology (stretching vs bending-dominated structures), architectural porosity and material they are made of. However, it is challenging to accurately predict its value in the case of AM-fabricated lattices, because of the geometrical mismatch with the as-designed structures. As such, it is preferable to assess the stiffness experimentally, according to the ISO 13314 [93].

With respect to the performance of zirconium lattices in a biological environment, no prior knowledge is available and experimental testing is needed to assess their biocompatibility⁹. Following the Becker’s and regulatory bodies’ approach, biocompatibility is affected by both the material and the prosthesis’ features, including

⁹ As the testing for biocompatibility assessment is extensive, this topic has been developed in Appendix II.

its design and surface finish. Therefore, even though zirconium is biocompatible *per se*, L-PBF zirconium lattices may or may not be suitable for a biomedical application. For example, PMPs that populate L-PBF-generated surfaces (including those of lattices) might affect the biocompatibility of zirconium. Indeed, the presence of PMPs on biomedical PBF-fabricated devices (including those made in Ti6Al4V) is a cause of concern for many researchers [69, 138, 155-157].

As of today, there is no regulation that imposes precise surface quality standards for PBF- and AM-generated medical devices, nor on their geometry. The development of norms in these areas has been hindered by 1) the technical challenges in the measurement of both the geometry and the surface topography of such intricate objects; and 2) the lack of established methods for their characterization.

With respect to the geometrical characterisation of lattices, the technical challenges in measuring inaccessible PBF-generated features can be overcome by using X-CT. Some attempts for the characterisation of PBF-fabricated medical devices and porous features have indeed been made (notably by Du Plessis [71, 113-115]). Nevertheless, at the current stage, the dimensional characterisation of lattices is often performed manually (hence with lengthy operations) or by using specialistic software (with the related costs), whilst automatic analysis protocols are capable of characterising lattices that exhibit struts in only one orientation. A tool that allows the prompt assessment of the geometrical accuracy for lattices with struts in multiple orientations is still to be developed.

Regarding the topographical characterisation of PBF-generated surfaces, the majority of published methods is based on texture parameters, which require the decomposition of surface texture into “waviness” and “roughness”, whilst few protocols are feature-based (FBC). The characterization with texture parameters is inherently challenging for AM-generated surfaces, because of distinguishing between waviness and roughness is tricky. Furthermore, texture parameters cannot describe features that are typical of AM surfaces, like PMPs, weld tracks, etc. The FBC, instead, seems a promising approach to

characterise these surfaces, as it can identify and measure specific topographical formations, including PMPs. The topographical characterisation for AM (and PBF) surfaces is still at its infancy and limited work has been done on PBF lattice and porous structures, whilst effective methods for the functional characterisation of orthopaedic-aimed lattices are still to be developed.

Given the above, this thesis aims at addressing the knowledge gap by achieving the following objectives:

1. Investigate different L-PBF manufacturing process settings and suggest optimal L-PBF process settings to fabricate Zirconium lattices with suitable geometrical accuracy and structural integrity.
2. Assess the mechanical and biological performances of the structures fabricated with the optimal process settings in relation to the material's properties.
3. Develop a protocol to characterise the geometry of L-PBF lattice structures with an "orthopaedic-tailored" approach (notably aimed at measuring the struts and nodes size in the relevant directions, the architectural porosity, and at assessing the eventual presence of occluded architectural pores).
4. Develop a protocol to characterise the surface topography of L-PBF lattice structures with an "orthopaedic-tailored" approach (a method to detect and measure PMPs).

The work focuses on the octet lattice, a stretching-dominated structure with demonstrated positive outcomes in clinical trials.

Chapter 3

Materials and Methods

This chapter provides the methods adopted in the experimental campaign of this study. The chapter is organised in the following sections. The first describes the design of the lattice structure (§3.1). The second presents the Zr702 powder used in the study and details the Design Of Experiments (DOE) deployed in the optimisation study for the Zr702 lattice structures as well as the manufacturing conditions for printing Zr702 bulk disks with L-PBF (§3.2). Then, the criteria used in the L-PBF process optimisation for the 702Zr octet lattices are illustrated (§3.3) and the methods for the lattice characterisation are described (§3.4). The details of the mechanical tests and biological tests are given in §3.5 and §3.6, respectively. The protocols for the acquisition and characterisation of the surface topography of lattice structures are given in §3.7. Finally, §3.8 summarizes the methods used in the study and highlights how these allow to answer the research questions outlined in Chapter 1.

3.1 Design of lattice structures

As mentioned in Chapter 1, the octet lattice (Figure 13a) was selected as lattice topology. Indeed, the octet topology has been already applied in orthopaedics with positive clinical outcomes [10]. The unit cell size (UCS) and strut diameter (ϕ_s) of the octet microtruss were 1.1 mm and 0.200 mm, respectively. These were chosen to satisfy the design criteria relative to osseointegration and manufacturability proposed by Melancon *et al.* [10], as shown in Figure 13b. Designed (architectural) porosity P_A (%) and designed pore (ϕ_p , the maximum inscribed circle) – calculated with Equation 5 and Equation 6 – were 65% and 0.578 mm, respectively. The topological features of the designed octet are summarised on the diagram in Figure 13b by the red bullet D (D = target design).

Equation 5

$$P_A(\%) = 1.58 \times \left(\frac{\Phi_P}{L}\right)^3 \%$$

Equation 6

$$\Phi_P = L - \Phi_S$$

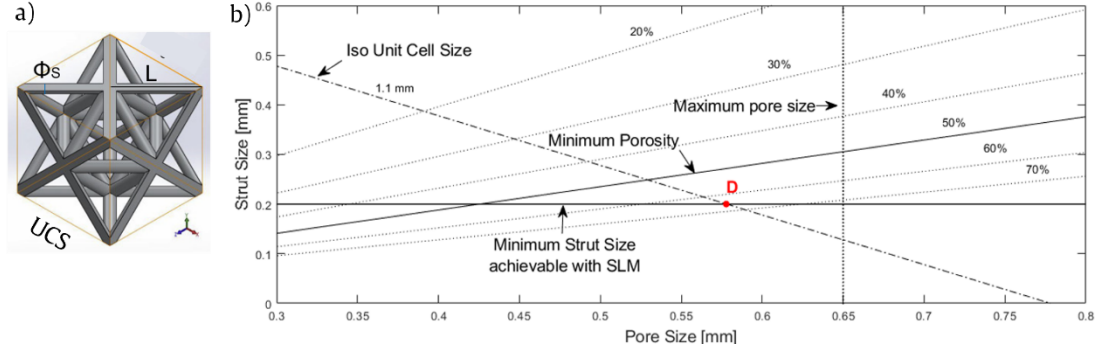


Figure 13 a) Octet lattice unit cell, characterised by the unit cell size (UCS) (or strut length, L) and the strut size (Φ_s); b) space design for the octet structure, defined by osseointegration and manufacturability constraints, on strut size, pore size, and architectural porosity. The red point D indicates the designed octet features adopted in this study.

3.2 Fabrication of lattices and bulk disks (L-PBF process conditions)

Lattice structures and bulk disks used in this study were fabricated *via* L-PBF (Concept Laser M2 Cusing SLM system) using the spherical atomised powder of Zr702 (nominal particle size 10–45 μm) supplied by TLS-Technik. Table 1 reports the chemical composition of the powder, as per supplier analysis.

Table 1 Chemical composition of the Zr702 powder supplied by TLS Technik used in this study.

Zr + Hf (%)	H (%)	Cr + Fe (%)	N (%)	C (%)	O (%)
99.80	0.001	Cr < 0.01 Fe 0.05	0.003	0.01	0.08

The metallic powder was also analysed in terms of particle size distribution at the University of Birmingham. The main indexes for particle size diameters (D_{10} , D_{50} and D_{90}) are shown in Table 2. These values indicate the particle size below which a specific percentage (10%, 50% and 90%) of the powder batch analysed is found, and are commonly used to characterise the powder size [166].

Table 2 Particle size indexes. The data were kindly shared by the University of Birmingham.

D10 (μm)	D50 (μm)	D90 (μm)
8.80	22.10	42.87

Figure 14 illustrates the results of the density distribution and cumulative distribution for the diameter of the powder.

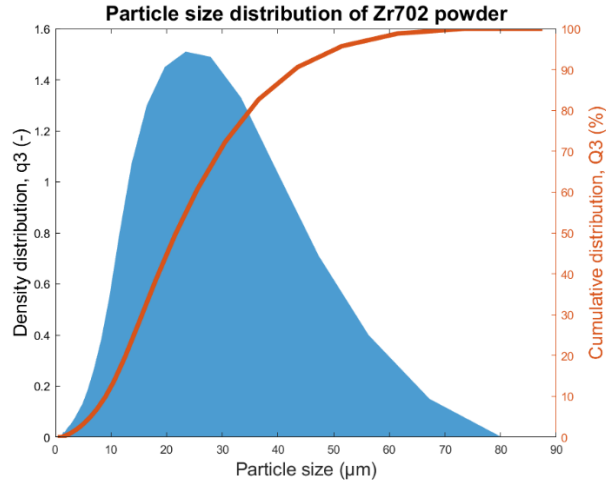


Figure 14 Results of the particle size distribution analysis for the Zr702 powder used in the study. Here, the cumulative distribution for the diameter ($Q3, \%$), and the density distribution ($q3, -$) for the diameter classes are plotted. The raw data were kindly provided by the University of Birmingham. The graph was elaborated by the author of this PhD thesis in MATLAB® with an in-house script.

The layer thickness (L-PBF parameter) was set to $30 \mu\text{m}$ in all the manufacturing trials.

Bulk disks were manufactured using a continuous laser power of 250 W, a scanning speed of 1500 mm/s and a beam compensation of 0.025 mm. The scanning strategy used for the bulk structures was the “island strategy” with an island size of 2 mm, and the pattern was rotated by 45° for each subsequent powder layer, as shown in Figure 15.

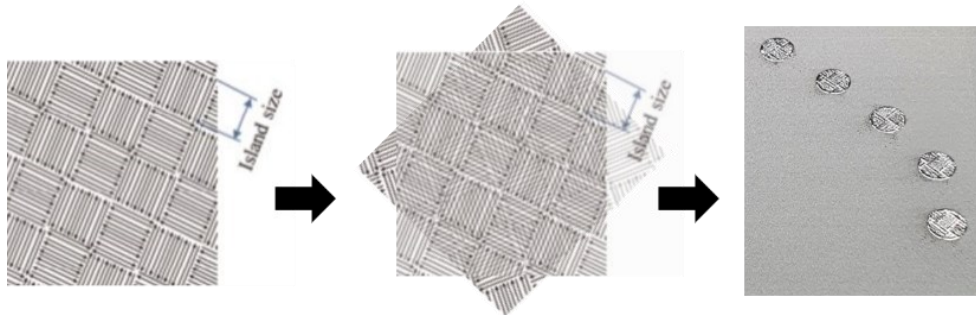


Figure 15 Illustration of the island strategy, which was used in the manufacturing of lattices with CX conditions and bulk cylinders. The island pattern was rotated by 45 degrees at all subsequent layers.

This scanning strategy was chosen because it allows to minimise the residual stresses built in the component during the manufacturing [106, 107].

3.2.1 Preliminary investigation of the process window for optimal process settings for lattice fabrication

Lattices were fabricated *via* L-PBF following the settings given above. For this study, the island strategy (5 mm length of each island) was used as scan strategy. Each island was rotated of 45 degrees between each layer. This strategy enhances the process by minimizing the residual stresses in the manufactured components.

Laser power (P), scanning speed (v_s) and hatch spacing (h_s) were varied on three levels, in the attempt to find an optimal setting to print the octet lattice. The power levels were 150 W, 200 W and 250 W; the scanning speed levels were 800 mm/s, 1400 mm/s and 2000 mm/s; the hatch spacing levels were 45 μm , 60 μm , and 75 μm .

The Design of Experiment (DOE) by Taguchi was chosen to reduce the number of process settings from twenty-seven to nine process conditions, which are shown in Table 3.

Two specimens for each process setting condition were fabricated, and samples were removed from the building platform by electrical discharge machining.

Table 3 L-PBF process settings used for the fabrication of lattice structures (C1-C9). P is the laser power, v_s is the scanning speed, and h_s is the hatch spacing.

Process Condition	P (W)	v_s (mm/s)	h_s (μm)
C1	150	800	45
C2	150	1400	60
C3	150	2000	75
C4	200	800	60
C5	200	1400	75
C6	200	2000	45
C7	250	800	75
C8	250	1400	45
C9	250	2000	60

3.2.2 Targeted investigation of process setting parameters with contour strategy (second optimisation study)

Following the first process parameters study, a second campaign was launched with the aim of increasing the geometrical accuracy of the fabricated lattices.

In the attempt to achieve better geometrical accuracy, the “contour strategy” was adopted as scanning strategy. The same strategy has been adopted by several researchers [2, 99, 108, 111] for the fabrication of lattice structures in various materials. An example of the contour path used in this study is shown in Figure 16.

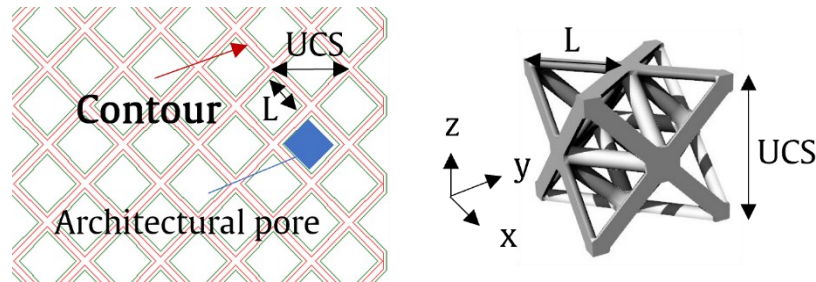


Figure 16 Schematic representation of the contour scanning strategy for the fabrication of Zr702 octet lattices. The red lines represent the contour scanning path for a specific layer in the powder bed in this study (screenshot taken from M2 Cusing System L-PBF machine interface).

The beam compensation (BC) was set at 0.05 mm, whilst the contour distance (CD) was varied on two levels: 0.050 mm and 0.075 mm. The laser power was kept constant (150 W), whilst the scanning speed was varied between 1400 mm/s and 1800 mm/s. The process setting conditions investigated in this campaign are summarised in Table 4.

Table 4 Process parameters relative to the conditions L1-L8, investigated for the second process investigation study. P is the laser power, v_s is the scanning speed, BC is the beam compensation, and CD is the contour distance.

Process condition	P (W)	v_s (mm/s)	BC (mm)	CD (mm)
L1	150	1400	0.05	0.050
L2	150	1600	0.05	0.050
L3	150	1700	0.05	0.050
L4	150	1800	0.05	0.050
L5	150	1400	0.05	0.075
L6	150	1600	0.05	0.075
L7	150	1700	0.05	0.075
L8	150	1800	0.05	0.075

3.3 L-PBF process optimization criteria for octet lattice manufacturing

The process optimization intended to pursue simultaneously: 1) geometric compliance of the manufactured lattice for proper bio-mechanical performances); 2) micro-structural lattice integrity); 3) macro-structural lattice integrity); 4) adequate quality of lattice surfaces (in a bio-mechanical perspective/to enhance biological performances and limit surface-related mechanical failure).

The criteria adopted for the process optimization are here listed in hierarchical order of importance:

- 1) Interconnected designed porosity P_A (%) greater than 50%, strut size as close as possible to the target design ($\phi_s \rightarrow 0.200$ mm) and no occluded architectural pores (*geometrical accuracy in a bio-mechanical perspective*)
- 2) Good lattice connectivity: few broken struts or through holes in nodes (*macro-structural integrity*)
- 3) Process-related porosity (internal porosity entrapped in the material, P_p (%)) lower than 0.5% (*micro-structural integrity*)
- 4) In the event that more than one process setting satisfies the previous criteria, the condition which leads to the lowest concentration possible of partially-melted particles (PMPs) on lattice struts and nodes is to be preferred (*surface quality*).

3.4 Lattice characterization with Non-Destructive Testing: characterization by X-rays Computed Tomography (X-CT)

3.4.1 X-rays Computed Tomography (X-CT) scanning of lattice structures

The X-rays Computed Tomography (X-CT) analysis was performed by the Advanced Materials Research Laboratory (AMRL) at the University of Strathclyde, using a Nikon XT H 225/320 LC.

For the analysis of C1-C9 lattices, one replica for each process condition was analysed by using an X-ray energy and current of 170 kV and 56 μ A, respectively. The final reconstructed spatial resolution (voxel size) was 10.95 μ m.

For the analysis of L1-L8 lattices, each sample was scanned by using an X-ray energy and current of 180 kV and 31 μ A, respectively. The final reconstructed spatial resolution (voxel size) was 7.25 μ m.

3.4.2 Image Processing: Levelling, Filtering and Segmentation

The X-CT data are a stack of non-binary images in which the levels of voxel intensity allow to identify specific phases of the scanned samples. The process by which the phases are identified is called “Image segmentation”, as single voxels are labelled (or segmented) as a specific class that composes the image. The reader who is unfamiliar with the X-CT data processing and analysis or the challenges encountered in the image segmentation of multi-phase images is referred to the review paper by Schluter *et al.* [167].

In the case of lattice structures, the expected phases are: 1) the material composing the lattice structure (high level of voxel intensity); 2) the air (or void) surrounding the material – that is the “architectural porosity” (or interconnected porosity) – characterised by low levels of voxel intensity; 3) the voids entrapped in the material – that are created during the manufacturing process (hereafter called “process-related porosity”) – also characterised by low levels of voxel intensity. In some cases, however, a fourth class may be identified: the “occluded architectural porosity”. This class would encompass those voxels with intermediate intensity and that would normally be classed as architectural porosity given their size and location with respect to the lattice structure but are not connected to the open porosity.

As mentioned above, the stacks of X-CT images need to be “segmented” or “labelled” into one of these three/four phases (called “Regions of Interest” ROIs) to allow the analysis and characterization of the manufactured lattices. The definitions of the “Lattice”, “Architectural Porosity”, “Process-related porosity” and “Occluded Architectural Porosity” ROIs are given in Table 5.

Table 5 Definition of the classes or Regions of Interest (ROIs) for the image segmentation of X-CT data

ROI	Definition
Lattice	Voxels with high level of intensity, identifying the material composing the lattice structure (struts and nodes)
Architectural porosity	Voxels with low levels of intensity, identifying the air (or void) surrounding the lattice and anyway within the volume occupied by the lattice phase if it were a bulk structure. Furthermore, this ROI is one single labelled object - that is the voxels belonging to this phase are connected to each other (6-connected) - and is in contact with the outer skin of the volume occupied by the lattice structure. This is the porosity intentionally introduced in the manufacturing for the production of lattice structure.
Process-related porosity	Voxels with low levels of intensity, identifying the voids entrapped in the material that are left over during the manufacturing process. These voxels are <i>not</i> in contact with the outer skin of the volume occupied by the lattice structure.
Occluded architectural porosity	Voxels with intermediate intensity and that would normally be classed as architectural porosity given their size and location within the lattice structure but are <i>not</i> connected to the open porosity. Strictly speaking, this is a sub-class of the Process-related porosity.

The workflow used in this study to segment the X-CT images was performed in ORS Dragonfly software, Version 2021 for Windows (Comet Technologies Canada Inc., Montreal, Canada; software available at <https://www.theobjects.com/dragonfly>).

A view of the raw X-CT data in the ORS Dragonfly software is given in Figure 17, whilst the procedure is illustrated in Figure 18. Briefly, the X-CT images relative to the lattices were imported in ORS Dragonfly software and levelled in a window of 28,187.5269-59,897.4946. All the voxels belonging to the apparent volume occupied by the lattice structure were labelled as “All” (in the example of Figure 17, the apparent volume is the volume of the cylinder). This ROI was then segmented with Otsu’s method to separate the foreground (that contains the voxels with the highest intensity, or the Lattice ROI) from the background (the voxels with the lowest intensity, or the Architectural porosity and the Process-related porosity).

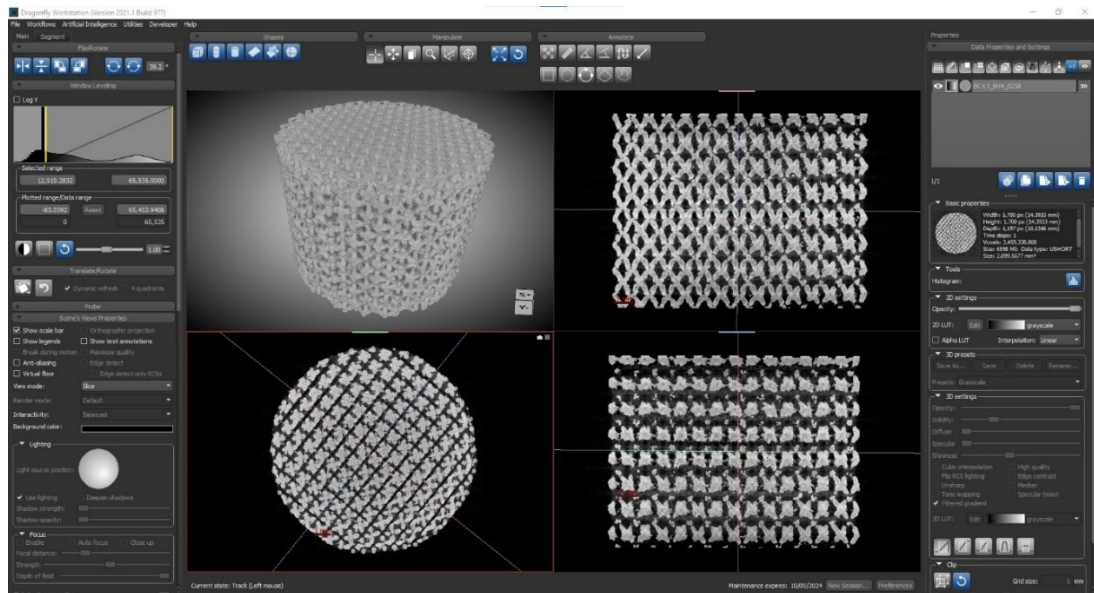


Figure 17 Overview of ORS Dragonfly software, which was used for the image processing of the raw X-CT data. The software enables the visualisation of X-CT images across three directions and move from section to section (or plane-by-plane) to follow the material distribution across the lattice. The main advantage of ORS Dragonfly is that it allows to view the volumetric representation of the X-CT data. This enabled the 3D view of the fabricated lattice structures at a glance.

This required the creation of a new ROI called “Shell”, which contains the “skin” of the volume occupied by the porous sample (in the example of Figure 17, the outer cylinder). The Boolean operations for the “Shell” are described in Figure 18. Moving forward in the segmentation workflow, BG voxels would be classed as “Open Porosity” if they were connected to the skin of the sample; the BG voxels which were *not* connected to the shell would be those entrapped in the material and would be classed as “Closed Porosity”. In mathematical operations, this is equivalent to take the BG and Shell ROIs and “keep” the “intersected labels” – for the Open Porosity- and “remove” the “intersected labels” – for the Closed Porosity, as shown in Figure 18.

Both the Open Porosity and the Closed porosity were subjected to a refinement. Such operations are commonly performed to improve the image segmentation [167].

The Open Porosity underwent a morphological operation called “Close”, that enabled to close holes of specific sizes. This operation is used to limit the noise errors that might occur in the Otsu’s thresholding and has the effect to (slightly) increase the ROI (dilatation). Here, the operation closed very small holes (Kernel size = 3) in 3D with a cubic shape,

which is equivalent to the closure of holes with a maximum diameter of 31 µm in the CX lattices (island strategy optimisation study) and of 21 µm in the LX lattices (contour strategy optimisation study). The so-determined ROI was finally called “Architectural porosity”.

Following a similar philosophy, the “Closed Porosity” ROI was refined by removing 6-connected process islands by voxel-count, with a minimum voxel count of 27 (from 3³), as recommended in the review work on X-CT for AM by Du Plessis [114]. This operation was performed to reduce the labelling of noise in the X-CT data as material voids. Assuming a cubic/spherical shape of the pores, thresholding the process islands to a minimum of 27 voxels is equivalent to exclude pores with size/diameter of ~21-31 µm, for the lattices LX and CX, respectively. The so-refined ROI was then called Process-related Porosity.

Eventual Occluded Porosity were isolated from the Process-related Porosity by selecting the single objects with a size of at least 200 µm. This value was selected by considering the nominal size of the lattice struts: an enclosed porosity bigger than the nominal strut size would be expected to create a through hole in the lattice structure, and therefore to be (ideally) connected to the architectural porosity ROI. The resulting segmentation was validated by visual analysis.

Finally, the ROI “Lattice” was identified by Boolean operation as a difference between the following ROIs (Equation 7):

Equation 7

$$All - P_A - P_P = Lattice$$

The ROI “Lattice” was then exported as a stack of binary images, to allow the geometrical characterisation of the lattice struts and nodes in MATLAB, as described in section §3.4.4.

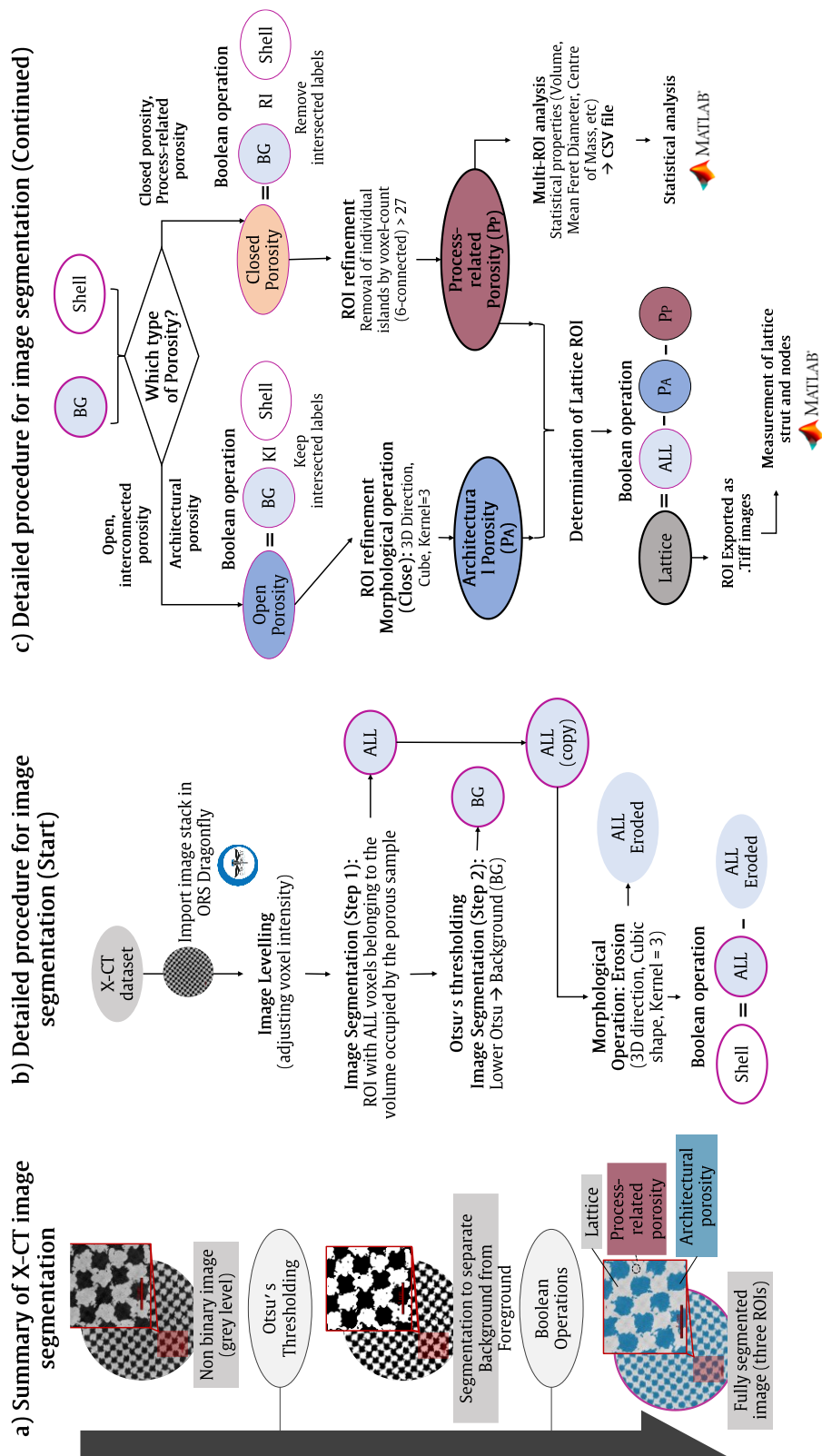


Figure 18 Procedure used to segment the images for analysing X-CT data. a) Summary of the image segmentation strategy, highlighting the main steps that allow to pass from a grey-level (non-binary image) to a fully segmented image with three Regions of Interest (ROIs): Lattice, Architectural Porosity and Process-related Porosity. The detailed procedure is illustrated in figure b) and c).

3.4.3 Characterization of the lattice structure: relative density, architectural porosity and process-related porosity from X-CT images

The number of voxels (v_X) belonging to each ROI (X) identified with the procedure described in the previous section (§3.4.2) were used to characterize the lattice structure in terms of relative density (ρ_{rel}), architectural porosity (P_A), process-related porosity (P_P), and eventual occluded porosity (P_O), with Equation 8, Equation 9, Equation 10 and Equation 11, respectively.

Equation 8

$$\rho_{rel} = \frac{v_{Lattice}}{v_{All}} \%$$

Equation 9

$$P_A = \frac{v_{Architectural\ Porosity}}{v_{All}} \%$$

Equation 10

$$P_P = \frac{v_{Closed\ Porosity}}{v_{Lattice}} \%$$

Equation 11

$$P_O = \frac{v_{Occluded\ Porosity}}{v_{Architectural\ Porosity}} \%$$

3.4.4 Image-based geometrical characterization of lattice structures (MATLAB Codes)

A series of MATLAB scripts (codes) were developed to perform an automatic geometrical characterization of the lattice struts and nodes on different directions. In particular, the algorithm measured the lattice struts that were parallel to the building platform (hereafter called “horizontal struts”) and those that were built at an angle of 45° with respect to the building platform (hereafter called “diagonal struts”). Moreover, the nodes were measured along the XY and XZ plane. The workflow is described in Figure 19 and the codes are available in Appendix I.

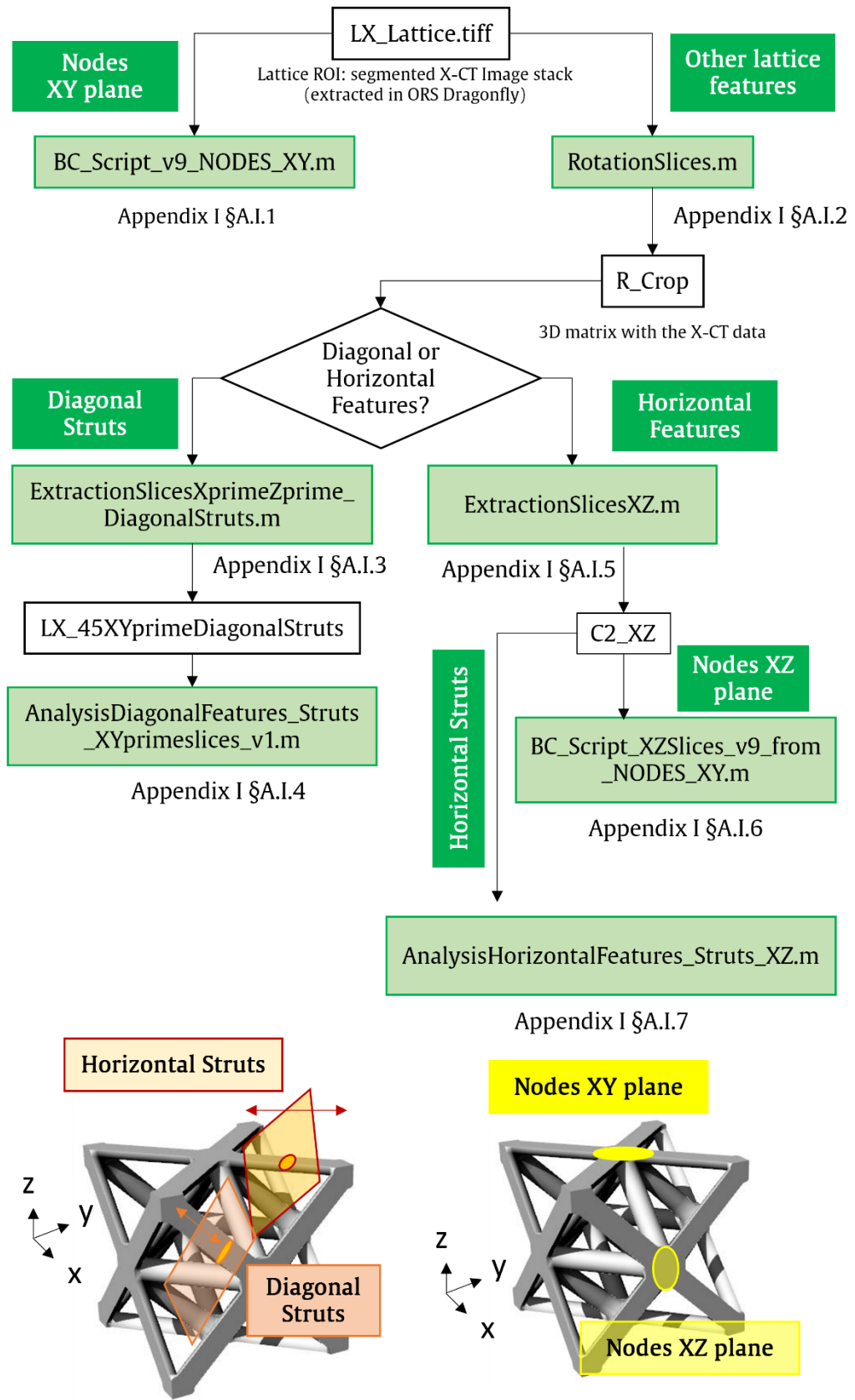


Figure 19 Workflow of the algorithms developed in MATLAB and illustration of horizontal and diagonal struts, nodes on the XY plane and on the XZ plane.

The Equivalent diameter was calculated for all the lattice features (struts and nodes), with Equation 12. The Feret diameters (including the orthogonal diameter) were measured in MATLAB with the function “feretPropertiesBEA”, which is reported in §A.I.8.

Equation 12

$$D_{equiv} = \sqrt{\frac{4A}{\pi}}$$

Where A is the measured surface area of the strut/node (isolated object in MATLAB).

3.5 Mechanical assessment

3.5.1 Mechanical test of L-PBF Zr702 lattice structures (compression test)

Zr702 lattice structures were tested in compression to assess their mechanical performance and evaluate their potential for orthopaedic applications. The characterisation of their elastic gradient, i.e. the elastic modulus of the lattice, is the main objective of these tests.

The L-PBF Zr702 lattice structure fabricated with conditions C2 was tested in compression following the prescriptions of ISO 13314 [93].

Three samples were used for the “preliminary test” of ISO 13314 [93], to obtain the stress-strain curve (until the densification stage was reached), calculate the quasi-elastic gradient of the lattice and the plateau stress σ_{Plt} , and calculate the loads for which the 20% and the 70% of the σ_{Plt} are generated.

Another five samples were tested in the “unloading test” of ISO 13314 [93] to derive the elastic gradient of the lattice structure.

The tested samples were cubic-like, with an edge of L =11 mm. The width and height of the samples were measured with a calliper before the test. The samples’ bases, which are the surfaces in contact with the machine platens, were coated with boron nitride to reduce the friction with the platens and minimise the risk of barrelling in the test.

The compression test was performed with the universal testing machine Z150 (ZwickRoell, Germany) at the Advanced Forming Research Centre (AFRC). The

displacement between the machine platens – which is recorded by the universal testing machine – was used to calculate the engineering strains, in compliance with the ISO 13314 [93]. Although relying on the displacement of the platens is not recommended for bulk materials tests, this procedure is accepted when testing porous metallic structures because their compliance is much greater than the platens, which enables for an accurate measurement of strains during the compression test, even in the elastic region, where the strains are “small”. The same protocol has been widely employed for the mechanical characterization of lattice structures in literature [15, 86, 94, 168, 169].

The preliminary test of ISO 13314 [93] was performed at a true strain rate of 10^{-3} s^{-1} . A constant strain rate has been also employed by others [170, 171]. Here, this choice was dictated by Zirconium being a strain rate sensitive material [172]. Using a small and constant true strain rate allows to eliminate the strain rate sensitivity factor from the test. The quasi-elastic gradient was used to determine the “zero strain” point as per ISO 13314 [93].

In the unloading test of ISO 13314 [93], five lattice samples were compressed with the following cyclic loading: compression up to a load of 23 kN, unloading down to 6.6 kN, and re-loading up to 30 kN. In this test, the constant true strain rate condition could not be applied due to the machine restrictions in cyclic loading. Instead, the compression test was performed at a constant travel speed of 0.01 mm/s, which is also recommended in the ISO 13314 [93]. The elastic gradient of the lattice was calculated from the secant line obtained from the hysteresis loop which occurs during unloading and subsequent loading, as per ISO 13314 standard [93].

3.5.2 Mechanical test for bulk material (compression test)

The compression test of L-PBF-manufactured Zr702 was performed with the main objective of characterising the material’s Young’s modulus. This would enable the comparison between the compressive elastic moduli of the material and the lattice.

The compression test here described was performed according to an original protocol. Such method was developed to overcome the challenges in the measurement of small strains (which is crucial for the determination of the Young's modulus) which the author experienced when performing the test as per National Physical Laboratory guidelines [173]. Indeed, when testing cylindrical samples with an aspect ratio (the ratio of the initial height to its diameter) of 1.8 – which is recommended in [173], samples sheared, invalidating the tests. Therefore, the sample's geometry was chosen to have a smaller aspect ratio, to avoid shearing. Finally, a cylindrical bulk sample (diameter of 12.69 mm and height of 4.4 mm, aspect ratio = 0.4) was subjected to a cyclic compression loading, composed of six steps as reported in Table 6. The test was performed at a travel speed of 0.01 mm/s, following the tests performed on Zr702 lattices and, therefore, the recommendations of ISO 13314 [93].

Table 6 Loads varied in the compressive cyclic loading mechanical test for the cylindrical bulk Zr702 sample.

Pre-load	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
100 N	91 kN	100 N	130 kN	100 N	156 kN	100 N

The sample featured a speckle pattern, to enable the use of Digital Image Correlation (DIC) analysis during the test. Briefly, DIC enables to track the displacement of single points throughout the test, in order to measure the instantaneous lengths and finally to calculate the strains. In practice, a camera is used to capture the speckle pattern throughout the test, and the images are then analysed to measure the instant lengths (l_i) over the entire duration of the compression test.

The DIC analysis was chosen to derive the strains accurately, and ultimately to enable for an accurate estimation of the elastic modulus of the material.

The data relative to the compression test (i.e., loads, time from the mechanical test machine, instant lengths over time from the DIC, and sample dimensions) were handled in MATLAB, with a self-developed code "CompressionTestDataAnalysis.m", which can be

found in Appendix I. This code ultimately allowed to derive the engineering stress vs strain curve for the material and estimate the elastic modulus of the material with different methods: by linear fitting and by chord method, as shown in §A.I.9.

3.6 Biological tests

The biological tests were selected following the analysis of the standards and the literature review, that can be found in the Appendix II.

3.6.1 Material preparation

Zirconium Lattice structures and solid bulk structures used in the biological experiments were sealed in sterilisation pouches and autoclaved to be sterilised at 121 °C for 22 minutes (Portable Steam Sterilizer, Prestige Medical).

For cell cultures, Lattices and Bulk samples were placed in single wells of sterile 24 multi-well plates (under sterile conditions) and covered with 1 ml of complete media (Dulbecco's modified Eagles media, DMEM, Dulbecco) for 30 minutes at room temperature, to allow protein adsorption onto the zirconium materials and favour the cell adhesion. The media was then removed before adding the cell suspension onto the samples.

3.6.2 Cell culture

Cell culture of MG-63 cells was prepared following the protocol developed by the Department of Biomedical Engineering at the University of Strathclyde. MG-63 cells were detached from their flask by removing the media, washing the cell layer with versene (two washings with 5 ml), and by adding 1 ml of trypsin. After 2 minutes (once cells were detached), 5 ml of complete media were added to the flask to stop the action of trypsin (which would otherwise digest the cells). The cells were finely dispersed in the media and counted with a haemocytometer (10 μ L of cellular solution were used). The solution was then adjusted to have a seeding density (number of cells per unit area) of 2×10^4 cells/cm².

3.6.3 MTT assay

The MTT assay¹⁰ is a colorimetric test that allows for the indirect counting of living cells. The MTT assay is a colorimetric test that allows for the indirect counting of living cells. The MTT assay developed in this study was inspired by the works of Varkey *et al.* [174] and of Jonitz *et al.* [175], who used MTT assay and WST-1 assay, respectively, to measure the metabolic activity and estimate the cell viability of osteoblasts. These are discussed in further detail in Appendix II (§A.II.5).

The protocol used was developed at the Department of Biomedical Engineering at the University of Strathclyde.

The MTT was dissolved in Phosphate Buffer Saline (PBS) pH 6.75 to a concentration of 10 mM. The solution was then filtered under the biological cabinet through a 0.2 µm filter as a sterilization procedure. The filter solution was stored up to two weeks at 4 °C in the dark. The MTT was added to the cell cultures in different quantities depending on the multi-well plate used, as shown in Table 7, to keep the ratio between the volume of MTT and the volume of the media in the cell culture constant at 5:20.

Table 7 Volumes of MTT and DMSO according to the well-plate used.

Multi-plate type	Type of <i>in vitro</i> test	Volume of media (µL)	Volume of MTT added (µL)	Volume of DMSO added to dissolve formazan (µL)
96 multi-well	MTT calibration curve	200	50	200
24 multi-well	MTT Assay for viability test on scaffolds; MTT Calibration curve	1000	250	1000

After adding the MTT solution to the wells, the plates were incubated at 37 °C for two hours. At the end of the reaction, the media was removed, and the formazan produced was dissolved in Dymetil sulfoxide (DMSO), in volumes that depend on the multi-well type

¹⁰ The MTT assay owes its name to the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) which is used as a reagent to measure the metabolic activity of cells. More details on the MTT assay and other colorimetric tests can be found in Appendix II, §A.II.5.

used, as shown in Table 7. The solution was mixed by pipetting vigorously to obtain an evenly coloured solution.

Finally, 200 μ L of formazan-DMSO solutions were transferred in a 96 multi-well for the absorbance measurement. The absorbances were measured at 540 nm with a MultiSkan GO (Thermo Scientific) microplate spectrophotometer.

The main steps of the protocol are illustrated in Figure 20.

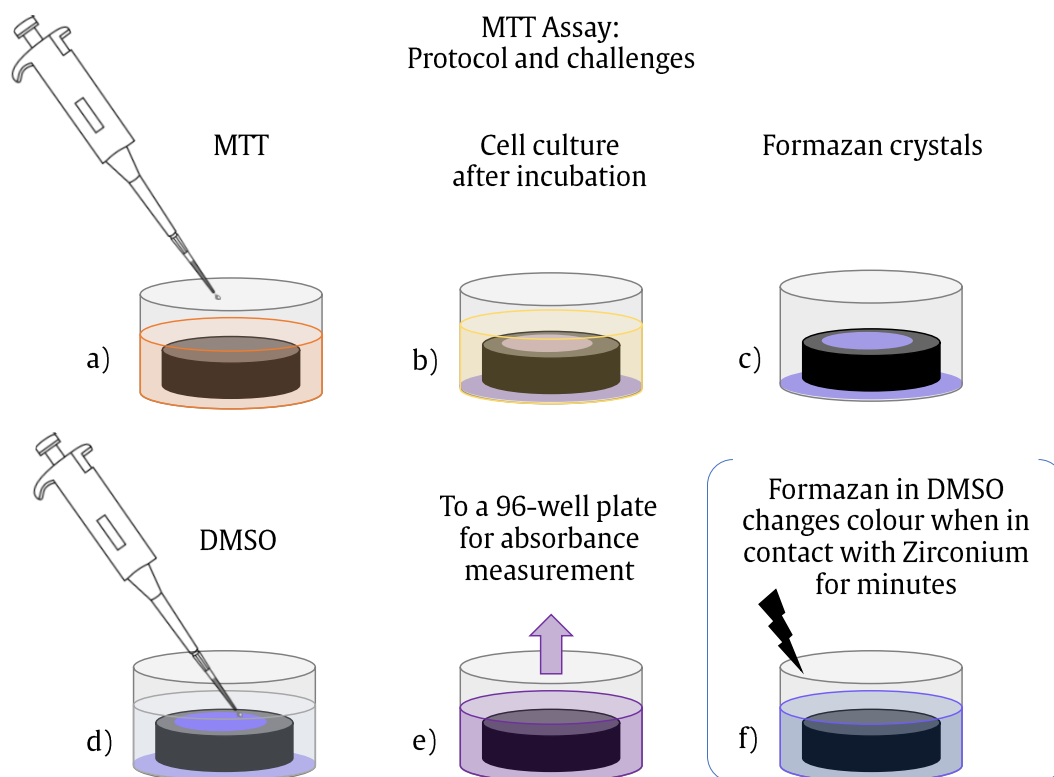


Figure 20 Illustration of the protocol used for the MTT Assay; a) introduction of the MTT (a tetrazolium salt) in the cell culture (in 24 well-plates) at specific times after the cell seeding; b) incubation of the well-plates for two hours, after which the formazan are formed (blue in colour) as shown in c); d) introduction of the solvent DMSO; e) mixing of the solution until an evenly coloured solution is obtained (purple or pink in colour depending on the amount of formazan obtained in the reaction) and extraction of 200 μ L to be analysed in a 96 well-plate for the absorbance measurement; f) illustrates the challenge of the MTT assay for zirconium substrates, as the solution formazan-DMSO changes in colour if left in contact with the metallic scaffolds for prolonged periods (minutes).

3.6.3 Fluorescence imaging

The staining with fluorescent markers (Iodidium Propide and Calcein AM) was used to visualise (and locate) living and dead cells onto the different substrates (tissue-culture plastic, zirconium bulk and zirconium lattice (C2), at 24 hours and 72 hours after the seeding.

The protocol, schematised in Figure 21, was developed by the Department of Biomedical Engineering at the University of Strathclyde and adapted (with minor changes) for the staining of cells on bulk and lattice zirconium.

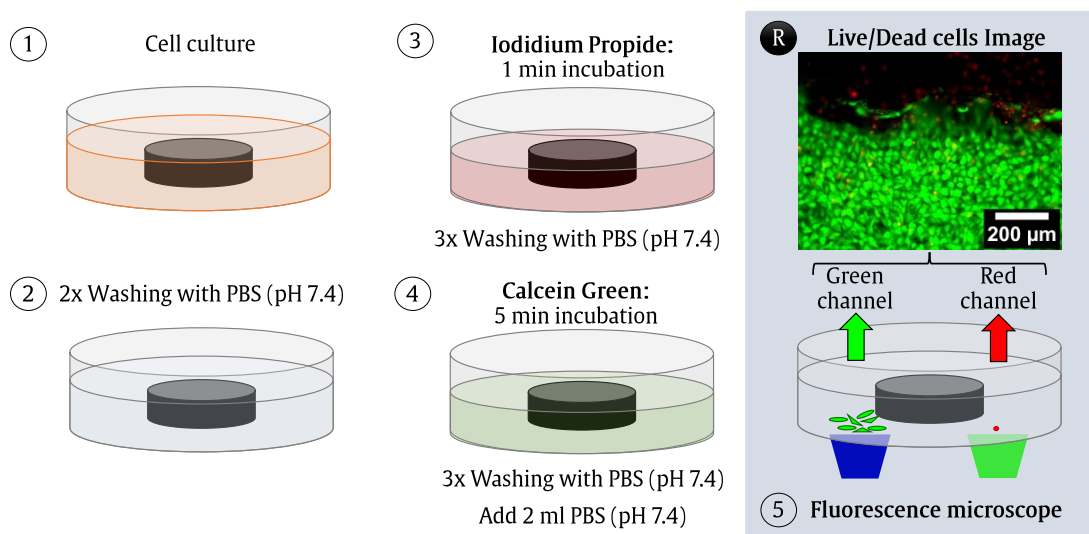


Figure 21 Illustration of the cell staining process with fluorescent markers (Iodidium Propide and Calcein AM) for live/dead discrimination. "R" stands for Result of the analysis.

At 24 hours (or 72 hours) from the seeding, the media were removed from the cell culture (Figure 21, step 1), and the cells were gently washed twice with PBS (pH 7.4) (Figure 21, step 2). Then, 1 ml of Iodidium Propide (20 µg/ml in PBS, pH 7.4) was added to the cell culture, and the system was incubated in the dark for one minute (at room temperature). The solution was withdrawn, and the cells were gently washed with PBS (pH 7.4) thrice (Figure 21, step 3). Calcein Green (1 ml, 5 µM in PBS, pH7.4) was added to the cell culture and let react with the cells for five minutes at room temperature. After removing the Calcein Green (hereafter called CG), three washings were carried out as for the Iodidium Propide (hereafter called IP). Finally, PBS (pH 7.4) was added before viewing the samples.

3.6.4 Statistical analysis of biological tests

In the analysis of the MTT test, one-way ANOVA and Games-Howell Post-Hoc test were performed in the comparisons between groups. The differences were considered statistically significant at p-values lower than 0.05.

3.7 Surface Characterization

3.7.1 Surface topography acquisition with Infinite Focus Variation Microscopy (Alicona)

The surface topography of lattice structures was measured with the Infinite Focus Variation Microscopy (Alicona Infinite Focus IFM G4, Bruker Alicona, UK) with an objective of 10x, under polarised light and ring illumination. Infinite Focus Variation Microscopy is a well-established instrument for the measurement of surface topography, and offers the advantage of being able to capture relatively rough surfaces over a large vertical range [117, 141], which makes it an ideal system for the measurement of L-PBF lattice structures.

3.7.2 Surface topography analysis with MountainsMap®

The surface topography of lattice structures was analysed in MountainsMap® 9 Expert (Digital Surf, Besançon, France).

MountainsMap® 9 Expert is a commercial software that is widely used in industry for topographical analyses and enables the characterisation of topographies acquired with areal measuring instruments, such as Focus Variation Microscopy.

It allows for the analysis of the surface topography according to international standards (for example, ISO 25178) as well as to develop *ad hoc* protocols. Moreover, it enables the programming and automation of the topographical analysis of surfaces, as the sequence of operations (algorithm) applied to a specific surface topography can be saved as a “Minidoc” to perform the topographical analysis on other surfaces in an automatic way, maintaining the same protocol.

MountainsMap® 9 Expert has additional modules that enable the analysis of specific topographical features. Because this part of the study was focused on the partially melted particles (PMPs) that adhere to the surfaces of additively manufactured parts, the Particle Analysis module was used in the attempt to detect and measure the PMPs.

The Particle Analysis module allows to detect different *features* that are present on the surface topography. The features can be detected with four different methods: the

threshold segmentation, the ISO 25178-2 watershed segmentation, the edge detection, and the circle detection. Each of these is described and reviewed in Table 8.

Table 8 Methods available in MountainsMap® for the detection of particles (including protruding particles and pores). The criticalities observed are referred to the specific problem under investigation in this study: the detection of partially melted particles (protruding particles) on additively manufactured lattice structures.

Detection Method	Description	Criticalities observed
Threshold segmentation	It detects particles using one or two thresholds.	The method works on particles that stand out clearly from a flat background, and on particles that have similar height.
Watershed segmentation	It fully segments the surface and detects contiguous dale or hill motifs using watershed segmentation as per ISO 25178-2.	The method works on regular or even backgrounds, whilst it fails on wavy and uneven backgrounds.
Edge detection	It detects features using watershed segmentation, after the application of an edge detection filter. It detects features on wavy or uneven backgrounds and works well also on features with different heights or that are not clearly separated (like connected particles).	The method is not able to discriminate between pores and protruding particles.
Circle detection	It detects small round particles and discriminates between protruding particles and pores.	The method works only on surfaces that exhibit circular particles which have similar dimensions.

From Table 8, it appears that the best method to detect partially melted particles on lattice structures is the edge detection method, although eventual superficial pores would be included in the detected features. The edge detection method is indeed the only method that works effectively on uneven and wavy backgrounds, which are typical of the additively manufactured lattice structures. Moreover, it offers the advantage of detecting features with varying sizes and at different heights in the surface topography, overcoming

the challenges of the circle detection and threshold segmentation. In brief, the edge detection is the method that offers the greatest flexibility in terms of background quality and feature sizes, among the ones currently available in the software, and has the best potential to detect partially melted particles on the topographies of lattice structures.

The edge detection method was tested against the other methods available in MountainsMap®, and it demonstrated its superiority in the feature detection on lattice surfaces (data not shown). Therefore, the detection method for PMPs on lattice structures that was developed here is based on this algorithm.

Before illustrating the final protocol developed in this study, it is worth illustrating the edge detection method settings in detail.

The edge detection may be tuned by pre-processing the topographical data and/or by defining pruning criteria on the detected particles.

The edge detection may be applied on the raw (unfiltered) or filtered (pre-processed) surface topography, depending on the features that need to be detected. Pre-processing filters smooth the topography by averaging the heights using neighbouring points and allow to filter out small features (very local peaks) in the edge detection operation. Instead, studying raw topographies allows to detect very small features. In the case of partially melted particles, it is desirable to detect even the smallest features in the topographical data, and therefore it is desirable to study the raw topography. However, processing raw data with the edge detection method may result in an over-segmentation of the topography, as any small variation on the topography is captured as a feature. For this reason, the edge detection is coupled with a “Wolf pruning” operation, that allows to refine the topographical segmentation according to filters that can be set up manually. This operation allows to tune the particle detection and impedes the over-segmentation of single features into multiple features by merging insignificant motifs into larger ones (Particle Analysis Guide).

Finally, the method developed in Mountains® for the detection and measurement of PMPs on additively manufactured lattice structures involved the steps described hereafter and is schematised in Figure 22.

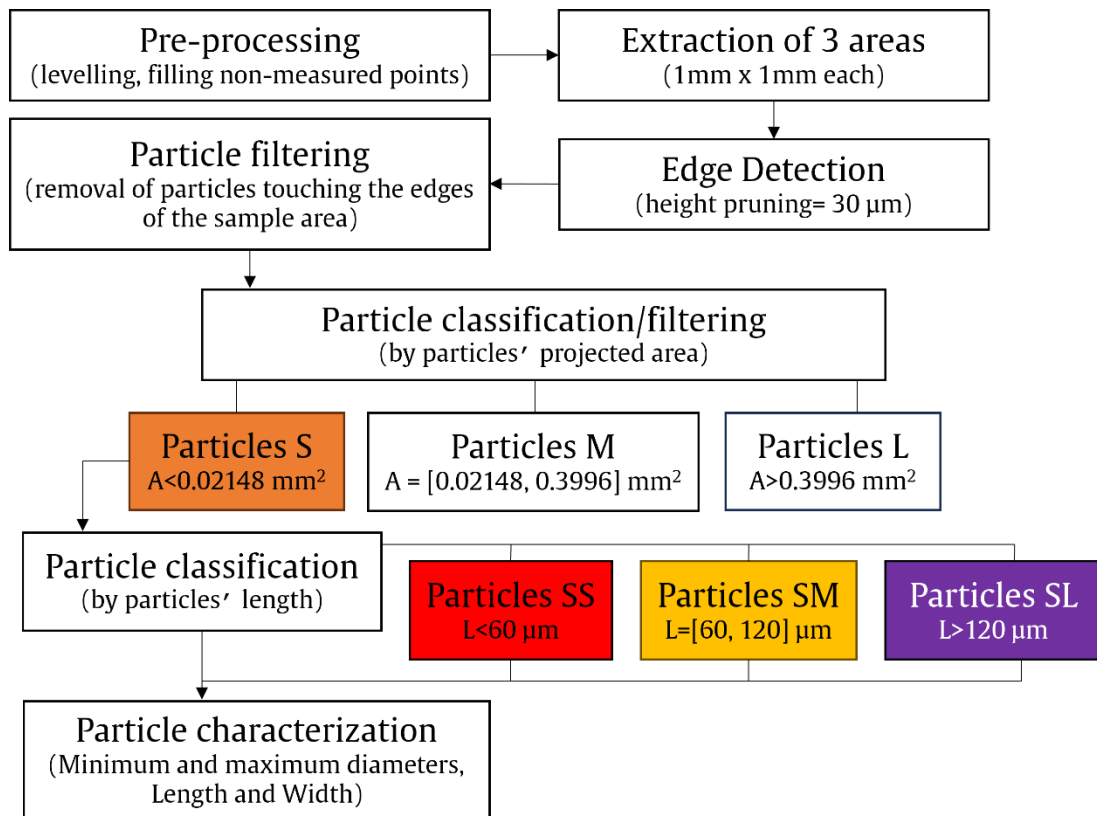


Figure 22 Flowchart of the particle detection and analysis method proposed in the study.

Pre-processing of the data consisted in levelling and removal of non-measured points. The levelling was implemented by least-squares mean plane subtraction, and non-measured points were filled with a smoothing operation. Three areas of 1mm x 1mm were extracted from the full data set and the particle detection was performed. The method of detection was the “Edge detection”, which allows to detect features with a watershed segmentation, after the application of an edge detection filter (Guide of Mountains® on Particle Analysis). The edge detection method was selected after comparing visually the results obtained with this and the other detection methods available in the software (i.e., threshold detection, watershed detection, and circle detection). The edge detection allowed a better and more accurate detection of particles on the surfaces compared with

the other methods. The surface topography was used in its raw form (unfiltered data) to allow the detection of the smallest features, and a height pruning of 30 μm was applied to prune the watershed segmentation. The chosen height pruning value coincides with the layer thickness of the powder used in the L-PBF process.

As mentioned earlier, the edge detection method segments the whole surface. In order to avoid capturing foreign/out-of-scope topographical features, the detected particles were classed into “Smaller” (S), “Medium” (M) and “Big” (B) particles according to the particles’ projected area:

S: Projected area < 0.02148 mm^2

M: Projected area $\in [0.02148 - 0.3996]$ mm^2

L: Projected area > 0.3996 mm^2

The “Smaller” particles (S class) were further subdivided into three groups (SS, SM, and SL), based on their length:

SS: Projected area < 0.02148 mm^2 AND Length < 60 μm

SM: Projected area < 0.02148 mm^2 AND Length $\in [60 - 120]$ μm

SL: Projected area < 0.02148 mm^2 AND Length > 120 μm

The range of these classes (SS, SM and SL) were defined based on the quality of the Zr702 powder used in the L-PBF lattice manufacturing, as the shape and size of PMPs strongly depend on the characteristics of the source material [139]. As single PMPs retain the size and shape of the metallic powder, SS was defined to contain detected particles with lengths lower than 60 μm , which corresponded to the 99% of cumulative distribution of the Zr702 powder (D99). SM and SL were defined to contain larger particles and would likely contain particle clusters and solidified molten droplets from the spatter that typically occurs in the L-PBF [139]. The ranges of the classes are visualised over the density distribution of the Zr702 powder in Figure 23.

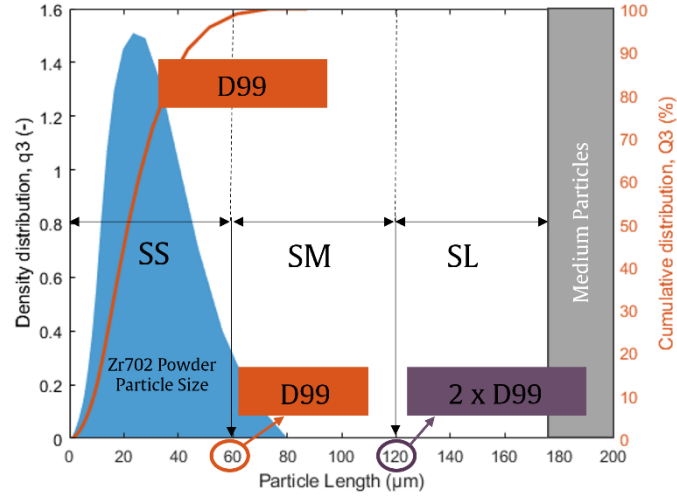


Figure 23 Ranges of particle lengths for the classes SS, SM and SL in comparison with the Zr702 metallic powder used in the L-PBF. The density distribution refers to the Zr702 powder. The ranges of the three classes were based on the length for which the 99% of cumulative distribution of the powder (D99) was observed, which is 60 μm . This value was used to define the upper limit of the SS class. The SM class ranges between D99 and its double.

The detected particles were further refined by excluding the particles on the edges of the area, in order to take out features that would extend beyond the area of observation. This allowed for measurement of only complete particles.

The length, width, maximum and minimum diameter of the detected SS, SM and SL particles were measured and extracted in .csv files. The statistical analysis was then performed in Excel.

The efficiency of the particle detection was evaluated by comparing the number of detected particles (P_D) and the undetected particles (P_U), the latter of which were counted manually in Fiji. The efficiency (η) of the method was calculated with Equation 13.

Equation 13

$$\eta = 1 - \frac{P_U}{P_D + P_U}$$

The particle density (D_P) was calculated with Equation 14.

Equation 14

$$D_P = \frac{P_D}{\text{area}} [\text{mm}^{-2}]$$

3.8 Summary of the methods

The methods used in this study are summarised in Figure 24. This scheme highlights how the adopted methods will allow to answer the research questions outlined in Chapter 1.

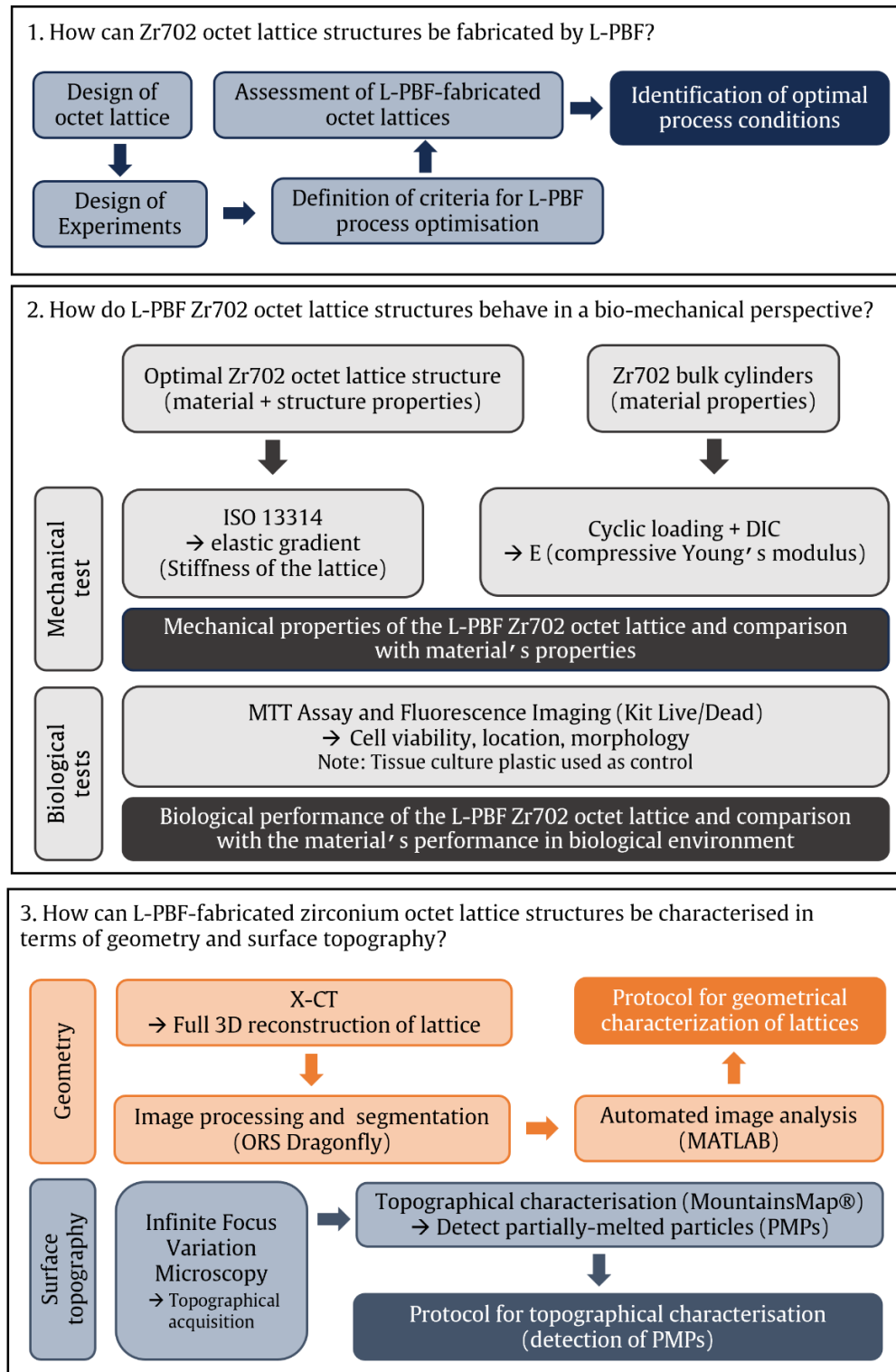


Figure 24 Scheme of methods in relations to the various objectives of the PhD study.

Chapter 4

Zirconium manufacturing by L-PBF and non-destructive characterization of lattices: Results and Discussion

4.1 Manufacturing of zirconium lattices and bulk cylinders by L-PBF

The manufacturing of the zirconium octet lattice structures and the bulk cylinders was performed using the Concept Laser M2 Cusing System L-PBF AM machine, and all the process conditions tested were suitable to manufacture full-volume macroscopically integer samples (i.e. no cracks were observed).

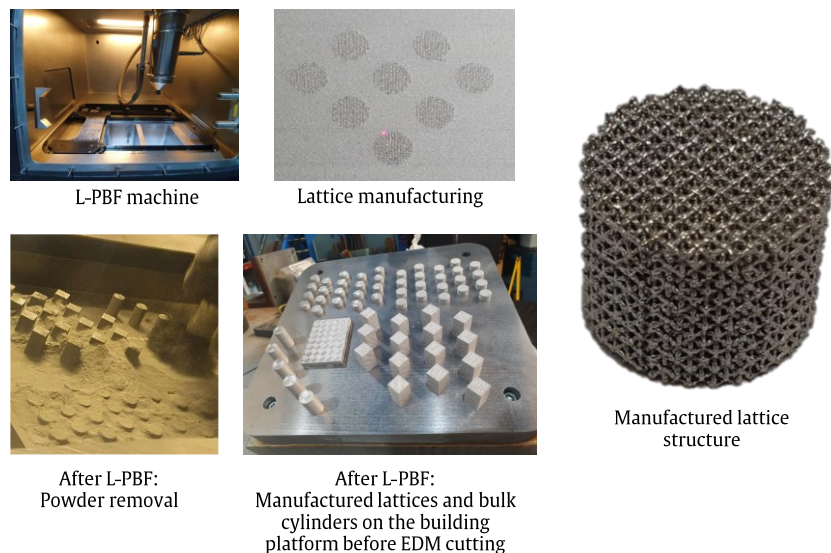


Figure 25 Manufacturing of lattice structures and bulk cylinders with different process parameters. The L-PBF machine used in the study (Concept Laser M2 Cusing SLM System) is shown on the top left; the lattice manufacturing is illustrated in the top centre of the figure; the key-post processing steps after the manufacturing are illustrated in the bottom of the figure, whilst a representative manufactured lattice structure is shown on the right.

Figure 25 illustrates the stages of the L-PBF manufacturing process and the samples obtained, with a particular focus on the lattice structure.

4.2 Identification of phases of lattice structures by X-rays Computed Tomography

The characterization by X-rays Computed Tomography (X-CT) of the manufactured lattices enabled for the assessment of the structures in terms of geometric compliance, macro-structural integrity (connectivity of struts and nodes), and micro-structural integrity (micro-pores that are internal to the material in the lattice struts and nodes).

The results of the image segmentation, that allowed to identify each phases composing the lattice structure's volume, are presented in this section.

The X-CT data were processed and segmented according to the protocols showed in §3.4.2. This allowed to label the voxels as "lattice" (V_L), "architectural pore" (V_{AP}), "process-related pore" (V_{PP}) and "occluded architectural pore" (V_{OP}), and therefore to identify the four different phases that compose the apparent volume of the lattice structures.

Table 9 and Table 10 report the distribution of these four phases in the lattices fabricated with the island strategy (manufacturing conditions C1-C9) and those fabricated with the contour strategy (manufacturing conditions L1-L8), respectively.

Table 9 Results of the X-CT data labelling relative to the lattices fabricated with the island strategy (C1-C9 manufacturing conditions, MC). The distribution of the phases (lattice, architectural pore, process related pore and occluded pore) that compose the volume occupied by the lattice structures is indicated by V_L , V_{AP} , V_{PP} and V_{OP} . The (laser) power, scanning speed (v_s) and hatch spacing (h_s) specify the lattice manufacturing conditions.

MC	Power (W)	v_s (mm/s)	h_s (μm)	V_L (%)	V_{AP} (%)	V_{PP} (%)	V_{OP} (%)	Total (%)
C1	150	800	45	62,49%	37,38%	0,13%	0,00%	100,00%
C2		1400	60	48,89%	50,91%	0,19%	0,00%	100,00%
C3		2000	75	35,38%	64,56%	0,05%	0,00%	100,00%
C4	200	800	60	69,94%	30,00%	0,00%	0,06%	100,00%
C5		1400	75	63,53%	36,32%	0,13%	0,02%	100,00%
C6		2000	45	60,22%	39,55%	0,23%	0,00%	100,00%
C7	250	800	75	85,38%	13,04%	1,09%	0,49%	100,00%
C8		1400	45	82,06%	16,87%	0,42%	0,65%	100,00%
C9		2000	60	64,93%	34,76%	0,26%	0,05%	100,00%

Table 10 Results of the lattice characterization by X-CT relative to the process conditions L1-L8 (contour strategy study). The lattice V_L , architectural pore V_{AP} and process-related pore V_{PP} phases are shown along with the scanning v_s and speed and contour distance (CD) which were varied in the optimisation study and characterise the manufacturing condition (MC).

MC	v_s (mm/s)	CD (μm)	V_L (%)	V_{AP} (%)	V_{PP} (%)	Total (%)
L1	1400	50	47.01%	52.92%	0,07%	100,00%
L2	1600		43.11%	56.84%	0,05%	100,00%
L3	1700		46.61%	53.33%	0,05%	100,00%
L4	1800		45.21%	54.73%	0,06%	100,00%
L5	1400	75	43.45%	56.43%	0,12%	100,00%
L6	1600		41.58%	58.31%	0,10%	100,00%
L7	1700		42.75%	57.16%	0,10%	100,00%
L8	1800		42.16%	57.74%	0,09%	100,00%

4.3 Fabrication of Zr702 lattices with the island strategy

The L-PBF optimisation study with the island strategy represents the first work on the fabrication of Zr702 lattice structures. As such, the process parameters (laser power P, scanning speed v_s and hatch spacing h_s) were varied along a relatively wide range. The aim of the study was to find the manufacturing condition(s) that would allow the fabrication of lattices with:

- A) an architectural porosity of at least 50%, strut size as close as possible to the target design (200 μm) and no occluded architectural pores (geometrical accuracy conditions)
- B) a process-related porosity of 0.5% upmost (micro-structural integrity condition) and
- C) a good strut and nodes connectivity (macro-structural integrity condition).

The analysis at X-CT enabled the characterisation of the fabricated lattices and the assessment of the lattice manufacturing in terms of geometric compliance, macro-structural integrity and micro-structural integrity.

Here, the results of relative density (ρ_{rel}), architectural porosity (P_A), process-related porosity (P_P), strut size (D_{equiv}) and structural integrity will be presented and discussed in relation to the process parameters used, and the optimal set of process parameters will be identified. The relative density, P_A , P_P and P_O were calculated from the labelled voxels (refer to Table 9) with Equation 8, Equation 9, Equation 10 and Equation 11 (refer to Chapter 4, §4.5.3) and are reported in Table 11. In the same table, the D_{equiv} represents the average strut size of horizontal and diagonal struts, as the strut orientation did not have a significant effect on the strut size (data not shown).

Table 11 Characterisation of relative density, architectural porosity, process-related porosity and strut size (average $\pm$ standard deviation) of lattices fabricated with the island strategy. The manufacturing conditions (MC) are determined by the laser power, scanning speed (v_s) and hatch spacing (h_s).

MC	Power (W)	v_s (mm/s)	h_s (μm)	Rho rel (%)	D_{equiv} (μm)	P_A (%)	P_P (%)	P_O (%)
C1	150	800	45	62,49%	345 ± 29	37,38%	0.20%	
C2		1400	60	48,89%	285 ± 38	50,91%	0.40%	
C3		2000	75	35,38%	256 ± 31	64,56%	0.16%	
C4	200	800	60	69,94%	403 ± 36	30,00%	0.01%	0.20%
C5		1400	75	63,53%	354 ± 31	36,32%	0.20%	0.06%
C6		2000	45	60,22%	346 ± 35	39,55%	0.37%	
C7	250	800	75	85,38%	504 ± 63	13,04%	1.26%	3.61%
C8		1400	45	82,06%	425 ± 52	16,87%	0.51%	3.70%
C9		2000	60	64,93%	349 ± 36	34,76%	0.40%	0.16%

As in can be seen from Table 11, the maximum architectural porosity (~65%) was achieved with the manufacturing condition C3 (laser power = 150 W; v_s = 2000 mm/s; h_s = 75 μm), whilst the lowest (~13%) was found in the lattice fabricated with the condition C7 (laser power = 250 W; v_s = 800 mm/s; h_s = 75 μm). The average strut size varied from 256 μm (condition C3) to 504 μm (condition C7). The maximum and minimum values of relative density and the strut size were achieved with the same manufacturing conditions (C7 and C3 conditions, respectively).

The P_P varied between 0.01% (condition C4) and 1.26% (condition C7) and occluded architectural pores P_O were found in the lattices fabricated with the conditions C4, C5 and

C7-C9, with C7 and C8 resulting in the lattices with the highest concentration of occluded pores ($P_0 = 3.61\text{-}3.70\%$).

4.3.1 The effect of the process parameters on the lattice features

In this section, the effects of laser power, scanning speed and hatch spacing on the lattice features - like the architectural porosity, strut size (upon which the relative density directly depends), and process-related porosity - are investigated.

The findings are organised into the following subsections: §5.3.3.1 presents the influence of the process parameters on the strut size, the architectural porosity, and the occluded porosity, which are the main features which determine the lattice *geometrical accuracy*; §5.3.3.2 discusses the effects of the manufacturing conditions on the process-related porosity and the lattice struts and nodes connectivity, which are connected to the lattice *micro-structural and macro-structural integrity*.

4.3.1.1 Process parameters that affect the geometrical accuracy of lattices

As discussed earlier, the geometrical accuracy of lattices is directly related to the lattice strut size, relative density, and architectural porosity. As the relative density (ρ_{rel}) is directly dependent on the strut size (if the strut size decreases, the relative density decreases and vice versa), the discussion on the influence of the process parameters on the relative density is not addressed to avoid redundancy (the conclusions on the strut size-process parameters relation can be directly extended to the relative density).

Figure 26 shows the trends of P_A and strut size with laser power (P) and scanning speed (v_s). As it can be seen from Figure 26a, the P_A tends to increase for increasing scanning speeds (v_s), whilst it tends to decrease with increasing laser power (P). The opposite trend is observed for the strut size (Figure 26b). Neither P_A nor the strut size were significantly affected by h_s .

For $v_s=1400$ mm/s (conditions C2, C5, and C8), P_A decreased by 15% and a further 19% when the power (P) was increased from 150 W to 200 W and from 200 W to 250 W, respectively. At the same scanning speed ($v_s=1400$ mm/s), the strut size increased by 24%

and by 20% when P was raised from 150 W to 200 W and from 200 W to 250 W, respectively. Overall, the increase of P from 150 W to 250 W was associated with a reduction of 34% of architectural porosity and a +49% strut thickening.

A similar trend was found at $v_s=800$ mm/s (conditions C1, C4, and C7), for which the rise in P (from 150 W to 250 W) led to a strut thickening of 46% and a reduction of architectural porosity of 24%.

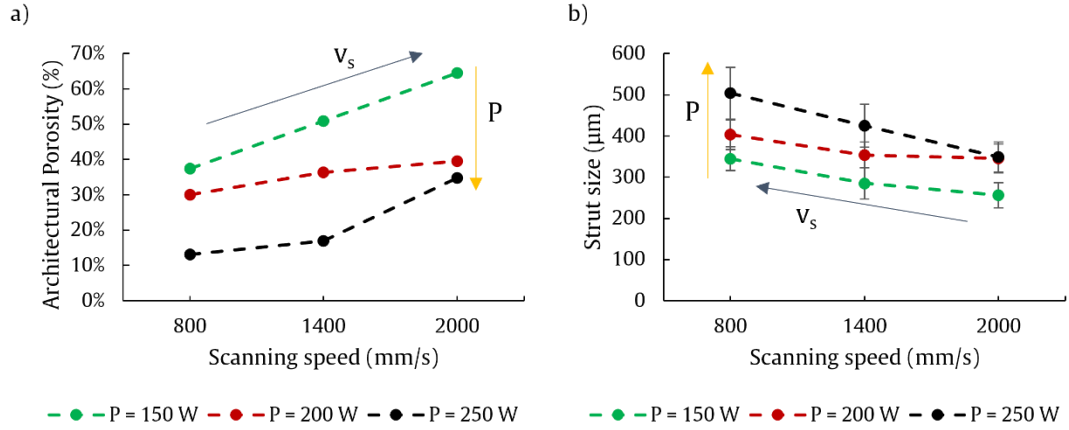


Figure 26 Effect of laser power and scanning speed on (a) the architectural porosity P_A and (b) on the strut size of lattice structures.

However, at $v_s=2000$ mm/s (conditions C3, C6, C9), the strut thickness increased by 35% when P increased from 150 W to 200 W, but no further thickening was observed when P was increased to 250 W. The trend was reflected (in the opposite way) in the architectural porosity, which decreased by 25% when the power was increased from 150 W to 200 W, and further decreased by just ~5% when the power was further increased to 250 W. This result seems to suggest that at high scanning speeds, raising the level of laser power from moderate to high does not have a significant effect on the strut size and the P_A . From the analysis of the strut size-energy density¹¹ (E_d , J/mm²) – which is reported in Figure 27 – the author concluded that the substantially equal strut size obtained with the manufacturing conditions C6 and C9 is likely due to the similar energy density that was

¹¹ The energy density E_d (J/mm²) is defined as the ratio between the laser power and the product of the scanning speed and the hatch spacing.

applied to the powder bed ($E_d = 2.1\text{--}2.2 \text{ J/mm}^2$). Indeed, the increase in laser power (+50 W, from C6 to C9) was counterbalanced by a decrease in hatch spacing ($-15 \text{ }\mu\text{m}$, from C6 to C9), which finally maintains the energy density almost constant.

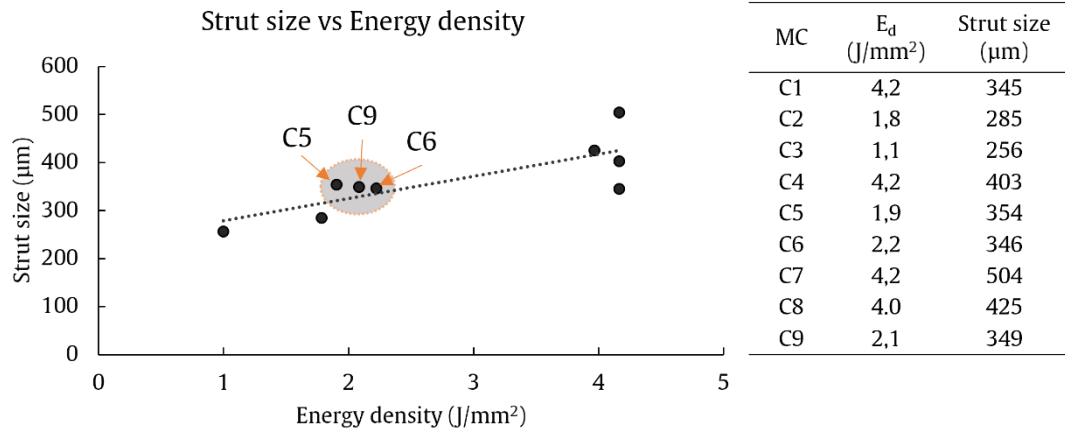


Figure 27 Average strut size against the energy density. The table on the right reports the numerical data. The conditions C5, C6 and C9, highlighted in the graph, were characterized by similar energy densities and average strut size.

On the other hand, at $P=200 \text{ W}$ (conditions C4, C5, and C6), it was observed that the average strut size decreased to $346 \text{ }\mu\text{m}$ following an asymptotic curve when the scanning speed was increased (refer to the red curve in Figure 26b). The same tendency had been reported by Qiu *et al.* [102], who worked on TiNi lattices and found that when a laser power of 400 W (the highest level in their study) was applied, the scanning speed did not significantly affect the strut size when it was varied between $3000\text{--}7000 \text{ mm/s}$. Considering the high laser power and scanning speeds involved, the authors attributed the invariability of the strut size for increasing scanning speed to the instabilities in the meltpool that originate in that speed range. In the case investigated here, however, the author believes that the substantially equal strut sizes of C5 and C6 is still due to the similar levels of applied energy density, as it can be appreciated in Figure 27. The effect of the energy density (therefore, the combined effect of P , v_s and h_s) on the strut size was proven by Tan *et al.* [2], who found that the lower the E_d , the smaller the strut size. Whilst Figure 27 demonstrates that the general trend was respected for the range of energy density investigated in this study, the author would like to highlight that the E_d represents

the third factor beside P and v_s for the strut size (and, consequently, the P_A). Whilst the correlation between E_d and strut size is rather strong, the spread of strut sizes for the $E_d \sim 4 \text{ J/mm}^2$ calls for caution in the use of E_d for process optimisation.

Overall, the results show that P_A decreases and the strut size increases with increasing laser power (P), whilst they exhibit the opposite trend with increasing scanning speed (v_s). These tendencies are in line with those reported by Qiu *et al.* [102] and Salem *et al.* [99]. The analysis of variance performed in Minitab® confirmed that P and v_s are significant factors for P_A (the p-value was 0.008 and 0.018 for P and v_s , respectively), whilst h_s did not show significant effect on P_A (p-value>0.05). Nevertheless, the E_d - a parameter that depends on P , v_s and h_s - showed a positive trend for the strut size: the higher the E_d , the bigger the strut size (and the lowest the P_A).

The last element that alters the geometrical integrity is the presence of occluded pores, P_O . Figure 28 shows the scatter plots of P_O against the individual process parameters and the E_d . The results seem to suggest that, within the investigated levels of laser power, the P_O tend to increase with increasing laser power (Figure 28a), and that $P=150 \text{ W}$ led to fully connected architectural pores (i.e., $P_O=0.00\%$). Nevertheless, there is great variability in the P_O when a single process parameter (P , v_s , and h_s) or E_d are varied. For example, for $P=250 \text{ W}$, the maximum investigated laser power of this study, the P_O ranged between 0.16% and 3.70% (Figure 28a). At the scanning speed 800 mm/s and 1400 mm/s, the P_O varied between 0.00% (no occluded pores) to the highest P_O measured in the lattices (3.61% and 3.71%, respectively). Indeed, raising the v_s from 800 mm/s to 1400 mm/s did not affect the average P_O (in black in Figure 28b), and only $v_s=2000 \text{ mm/s}$ seemed to minimise the P_O . Moreover, for all the levels of v_s and h_s investigated in this study, there was one lattice with $P_O = 0.00\%$ (refer to Figure 28b-c).

The observations made on Figure 28 and the statistical analysis performed in Minitab® allow to conclude that no individual process parameters (nor E_d) within the investigated levels is a significant factor for P_O .

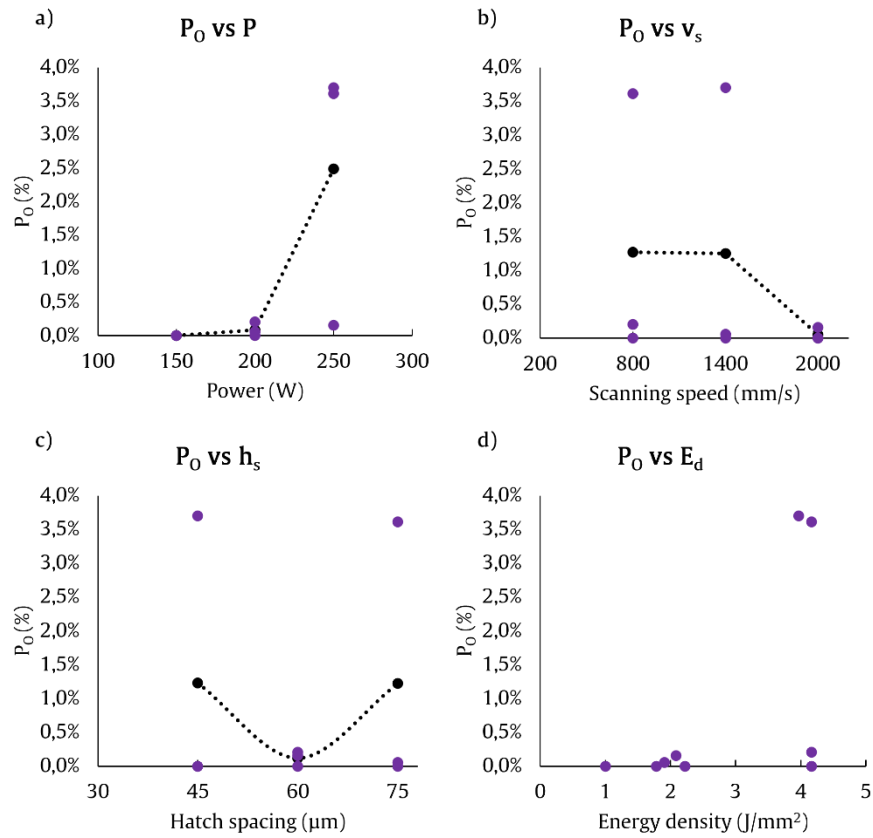


Figure 28 Occluded pores (P_O) with a) the laser power; b) the scanning speed; c) the hatch spacing and d) the energy density. The average values and the trend lines are in black.

The P_O was then plotted against the strut size and the architectural porosity P_A , in the attempt to find “safety levels” that would guarantee zero P_O (that is, that the architectural porosity is well connected in the whole lattice volume). As it can be seen from the scatter plots of Figure 32a-b, P_O exhibited a monotone behaviour with both the strut size and P_A . Importantly, both the strut size and P_A presented a threshold value beyond which the P_O were zero: indeed, the lattices that were characterised by struts sizes $< 345 \mu\text{m}$ and $P_A > 40\%$ showed no P_O . These thresholds, together with the designed strut size (200 μm) and P_A (65%), allowed to define the “Zero P_O ” regions, which represent the safety levels to avoid P_O . These regions are highlighted in the graphs (Figure 32a-b).

The safety levels here identified are specific to the designed lattice of this study (i.e. octet lattice with a unit cell size of 1.1 mm and a nominal strut size of 200 μm), and the results are not applied directly to other lattice topologies or geometries (especially if the unit cell

size is smaller than 1.1 mm). Despite the limited exploitability of the so-determined safety levels, defining the “zero P_0 ” regions based on the strut size and P_A offers some advantages in the process optimisation of lattice structures. First, this approach gives clear indications on how to avoid the generation of P_0 (i.e. strut and P_A should be kept in specific ranges) and, second, these rules are based on lattice features (strut size and P_A) that are significantly affected by specific L-PBF process parameters (P , v_s and E_d , as demonstrated above). Therefore, the control on P_0 is still related to the manufacturing, bypassing the issue of P_0 not being significantly affected by the investigated process parameters. The third consideration is that the safety rule is based on a rather intuitive principle: if the strut size is minimised and kept under specific levels, the architectural porosity is maximised and is more likely to be fully connected.

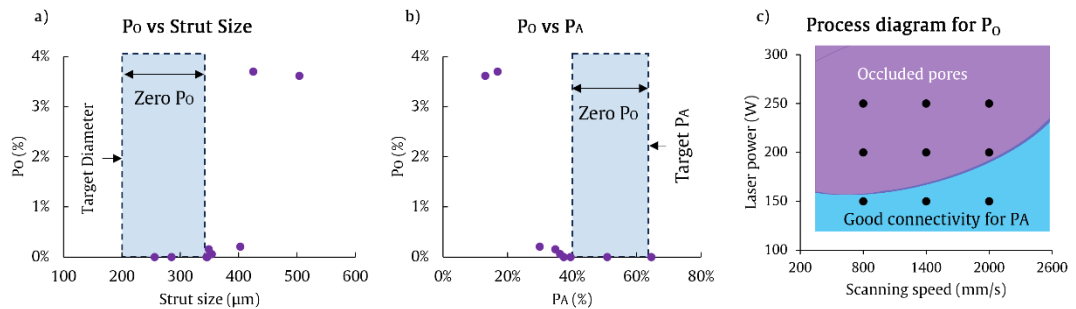


Figure 29 Occluded porosity (P_0) against a) the strut size and b) the architectural porosity; c) Process diagram for P_0 defined by the laser power and the scanning speed, in which each point corresponds to a tested manufacturing condition (MC). The diagram is divided into two areas: the conditions which led to occluded pores (in purple) and those who led to good connectivity for P_A .

As mentioned above, it is possible (and desirable) to link the P_0 back to the manufacturing. The process diagram for P_0 in Figure 32c has been drafted with this objective. The graph presents two regions, which are characterized by the presence (in purple) or absence (in blue) of P_0 . As it can be appreciated, all the MCs with the $P=250$ W exhibited P_0 , regardless of the scanning speed used. On the other hand, at the $P=150$ W, no P_0 was found, suggesting a good connectivity for P_A . Despite its simplicity, the process diagram allows to

quickly identify suitable process parameters for a good connectivity of the architectural porosity.

To summarise, the geometrical accuracy of lattices is affected by P , v_s and E_d .

The effect of P on the geometrical integrity is illustrated in Figure 30, which shows representative cross-sections of the lattices manufactured at the scanning speed $v_s=1400$ mm/s (conditions C2, C5 and C8). Minimizing P in the L-PBF process allows to fabricate lattices (in grey in the image) that are characterised by relatively small strut sizes and high architectural porosity (in blue in the image). Increasing the power leads to thickened lattice structures, which might still maintain a good interconnectivity of architectural porosity (observe the cross-section of the lattice relative to the C5 condition in Figure 30). However, high levels of P result in lattices becoming almost bulk structures and exhibiting a significant fraction of occluded architectural pores (marked in purple in the image).

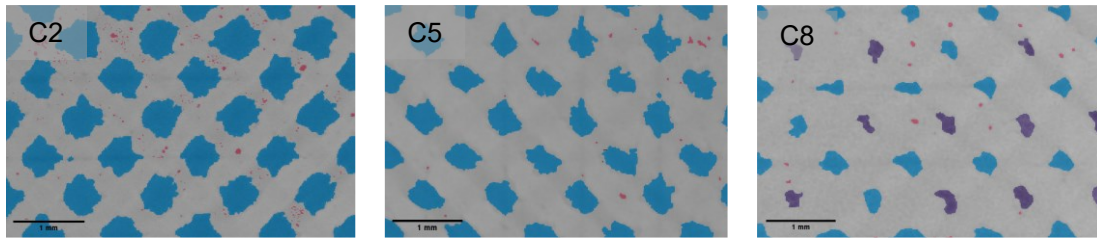


Figure 30 Representative cross-sections of lattices produced at the scanning speed of 1400 mm/s with condition C2 (on the left), C5 (in the middle), and C8 (on the right) for increasing laser power (from 150 W to 250 W). From the left to the right, the lattice (in grey) is progressively thickened, whilst the architectural porosity is decreased. For C8, a significant fraction of architectural porosity is occluded (in purple). The internal porosity (process-related pores) is coloured in coral red. Scale bar is 1 mm.

The scanning speed has the opposite effect on the geometrical accuracy with respect to P .

Therefore, the v_s should be maximised to increase the architectural porosity and to improve the geometrical accuracy, in general.

Finally, the E_d – the ratio of P over the product of v_s and h_s – should be minimised to minimise the strut size.

4.3.1.2 Process parameters that affect the micro-structural and macro-structural integrity of lattices

As mentioned above, the *micro-structural integrity* of the manufactured lattices may be undermined by excessive process-related porosity, P_p , which is the porosity enclosed within the material (i.e., within the lattice struts and nodes). Therefore, it is desirable to understand the influence of the L-PBF parameters on the P_p and to identify which process conditions lead to a better micro-structural integrity in lattices (low levels of P_p).

On the other hand, the *macro-structural integrity* of the lattices refers to the integrity (connectivity) of the struts and nodes, which is critical to the mechanical properties of the lattices and their bearing capacity. In contrast to the micro-structural integrity, which can be evaluated through a quantitative parameter (the P_p), the macro-structural integrity cannot be evaluated through simple figures. Indeed, the defects of macro-structural integrity result in volumes of material (the solidified metal in the lattice) being occupied by air, and the relative voxels (in the X-CT data) are therefore labelled (in blue) as connected architectural porosity (P_A). Because the macro-structural integrity was assessed only qualitatively, the impact of the process parameters on the macro-structural integrity will be presented and discussed in support to the discussion on the impact of the manufacturing on the *micro-structural integrity*.

The influence of the manufacturing parameters on the process-related porosity P_p was investigated with the scatter plots of P_p against the single process parameters (laser power P , scanning speed v_s and hatch spacing h_s) of Figure 31a-c. The process-related porosity P_p did not vary significantly with the single process parameters P , v_s , and h_s (p -values > 0.05). These results are in apparent contrast with the findings of Qiu *et al.* [102] and Read *et al.* [106], who found that P and v_s were significant factors for P_p . Nevertheless, the range of process parameters was either rather different from the levels tested in this study [106], or the DOE impeded to appreciate eventual interactions between the process parameters [102].

The findings of this work are, instead, well aligned with the study by Salem *et al.* [99], who reported an interaction between P and v_s . The same phenomenon can be observed here in Figure 31d-e. Let us analyse Figure 31d, which shows that the effect of P on P_p depended on the level of v_s . At the minimum scanning speed (800 mm/s), increasing the P from 150 W to 200 W led to a decrease in P_p ($\sim -0.2\%$), whilst a further increase in P led to a sharp increase in P_p ($\sim 1.3\%$). At the maximum scanning speed (2000 mm/s), instead, the increase in P from 150 W to 200 W led to an increase of $\sim 0.2\%$ in P_p , whilst a further rise in P did not result in a variation of P_p .

Considering the existing interaction between P and v_s , the author investigated the potential effect of E_d on P_p . Interestingly, the P_p did not show strong correlation with the energy density E_d (Figure 31f): although P_p seemed to increase with increasing E_d , the P_p in lattices manufactured at $E_d \sim 4 \text{ J/mm}^2$ varied extremely, from almost 0% to the maximum ($\sim 1.3\%$).

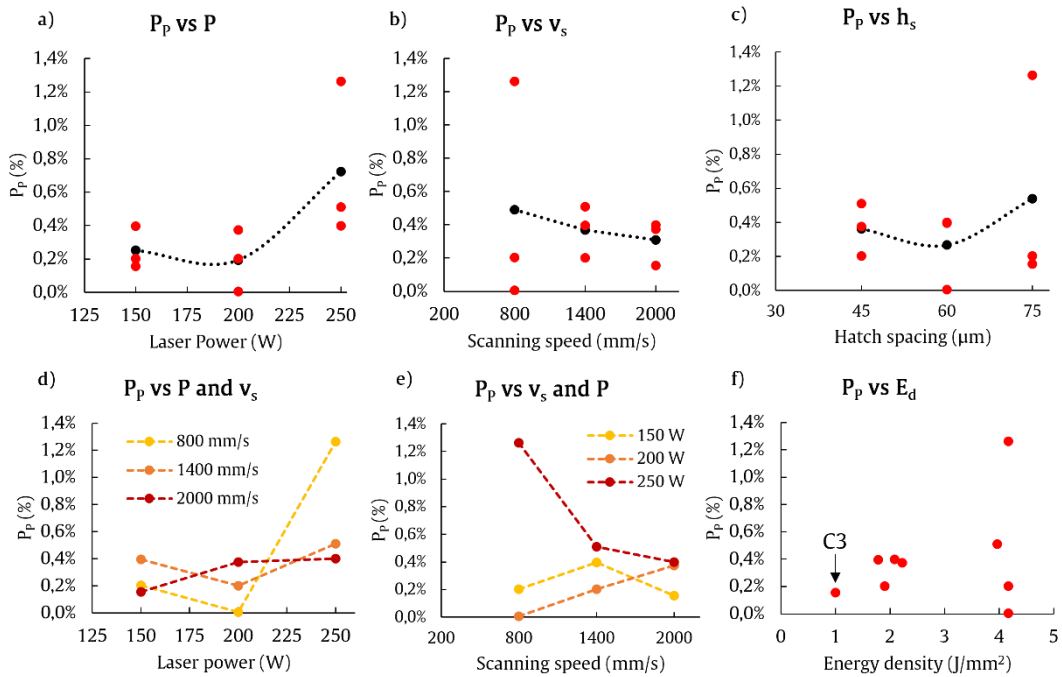


Figure 31 Scatter plots of process-related porosity (P_p) versus single process parameters: a) laser power, b) scanning speed, and c) hatch spacing. The black lines represent the trend lines of the average P_p with the process parameter of interest. Interaction plots of P_p with laser power and scanning speed (d-e); f) is the scatter plot of P_p with the energy density.

The analysis of the process diagram for P_p in Figure 32, which reports the P_p for each process condition (C1-C9) and the iso-surfaces for the P_p , confirms the combined effect of the laser power and scanning speed on the P_p and helps understanding the generation of P_p in the different manufacturing conditions.

The green regions in the centre of the diagram include the laser power and scanning speed levels that led to process-related porosities below the 0.05%, which is the upper acceptable level set in the optimisation study. This area is called “process window” and encompasses the manufacturing conditions that led to a good micro-structural integrity. These are: the low to moderate laser power combined with low to moderate scanning speed levels (C1, C4, and C5), for which $P_p \leq 0.2\%$ (in dark green in Figure 32); the low laser power and moderate scanning speed (C2), and the moderate to high laser powers combined with high scanning speeds (C6 and C9), for which the $P_p = 0.37\text{--}0.40\%$ (in light green in Figure 32). Above this region, the high levels of laser power combined with low to moderate scanning speeds (C7 and C8) arguably led to “keyholing” conditions, with P_p exceeding 0.5% (iso-surfaces coloured in red).

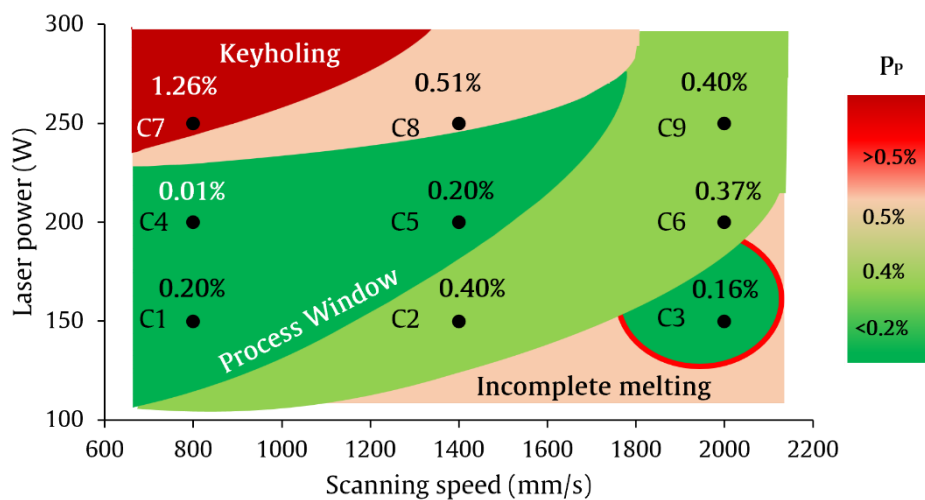


Figure 32 Process diagram defined by the laser power and scanning speed with iso-surfaces for the process-related porosity, P_p . The process window (highlighted in shades of green) is surrounded by areas characterised by higher process-related porosity due to keyholing (for high laser power and low scanning speed) or incomplete melting (low laser power and high scanning speed). The condition C3, circled in red, represents an apparent exception to the incomplete melting area.

Below the process window, the manufacturing is expected to enter the region of incomplete melting (also known as “balling”), in which the meltpool becomes unstable and the material solidifies in discontinuous tracks. This regime is due to insufficient energy input, and therefore P_p is expected to be rather significant (that is, exceeding 0.5%). The condition C3 (high laser power and high scanning speed) would seem to represent an exception to the process diagram, as its lattice exhibited a low level of P_p (0.16%). Nonetheless, the visual analysis of the labelled X-CT images and the 3D rendering of the lattice fabricated with C3 conditions revealed that the very low values of P_p were associated with a poor connectivity of the lattice structure (i.e. poor macro-structural integrity), rather than being related to an adequate and continuous powder melting across the struts. In Figure 33 are highlighted the through holes in the lattice nodes and the broken struts that interrupted the material continuity and jeopardised the macro-structural integrity the lattice fabricated with C3 condition.

The loss of the lattice connectivity and the irregular shape of the lattice struts are clear symptoms of incomplete powder melting condition in the process diagram. .

C3 is the combination of the minimum laser power, and the maxima scanning speed and hatch spacing tested in this study. As such, this process setting is characterised by the lowest energy density (1 J/mm^2) inputted to the powder bed (see Figure 31f).

This finding allows to draw three crucial conclusions. First, that such level of energy density is inadequate to melt the material in the full layer thickness. This hypothesis is corroborated by the study of by Aristizabal *et al.* [7], who indicated that the same level of energy density (realised with a $P=100 \text{ W}$, $v_s=2500 \text{ mm/s}$ and $h_s=80 \text{ }\mu\text{m}$) was insufficient to fully consolidate Zr702 bulk samples (the P_p varied between 0.6% and 1.1% depending on the specific manufacturing parameters).

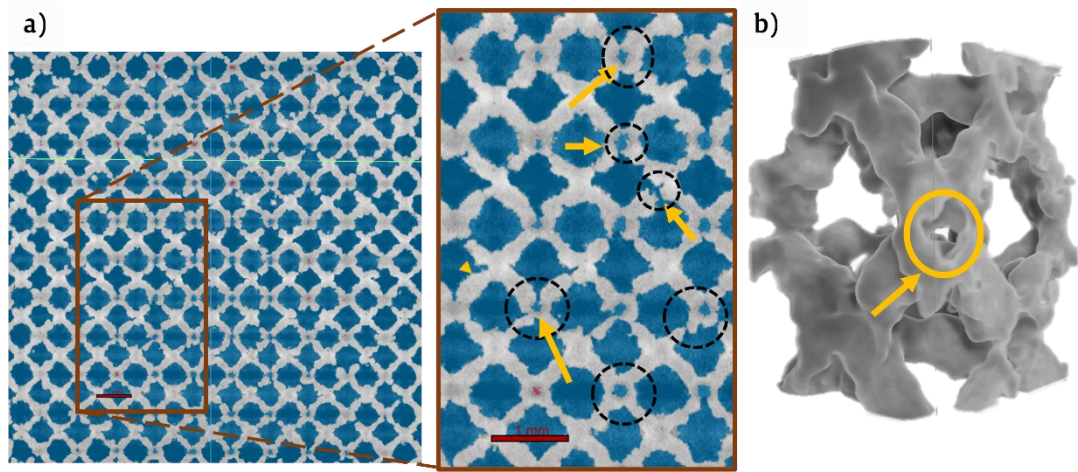


Figure 33 Cross-section of lattice fabricated with condition C3, exhibiting low process-related porosity (labelled in red) but poor macro-structural integrity (a). The orange arrows highlight the areas where the strut connectivity is lost, whilst the circles indicate through-holes in the nodes. The 3D rendering of the same lattice on a single unit cell (b) clearly shows the detrimental impact of these defects on the lattice structural integrity.

Second, characterizing the sole P_p without assessing the lattice connectivity might lead to gross error in the L-PBF optimisation for lattice structure: from the scatter plot of P_p against E_d in Figure 31e, the data seem to suggest that the lower the E_d , the better. However, the visual analysis performed on the macro-structural integrity allowed to spot that the low levels of P_p obtained at the minimum E_d represented only a Pyrrhic victory, obtained at the expense of the lattice connectivity.

Third, whilst the considerations made on the geometrical accuracy of the L-PBF lattices suggested to minimise the laser power and maximise the scanning speed, the analysis of the macro-structural integrity advises against such optimisation strategy. Indeed, elevated v_s combines with low P may lead to meltpool instabilities during the manufacturing and might result in lattice structures with poor macro-structural integrity.

Overall, the macrostructural and microstructural integrity of the lattices is affected by the combination of P and v_s . Whilst the E_d can often explain the levels of internal microporosity (P_p) and the quality of the lattice connectivity, however, the author suggests caution in the exploitation of such parameter for the process optimisation.

This study also demonstrates the need to characterise both the P_p and the macro-structural integrity, and to consider them together, when searching for a suitable process window to

manufacture lattice structures for load-bearing applications, such as orthopaedic implants.

4.3.2 The optimal parameters for the fabrication of Zr702 with the island strategy

The aim of the study was to find the manufacturing condition(s) that would allow the fabrication of lattices with:

- A) an architectural porosity of at least 50%, a strut size as close as possible to the target design (200 μm) and no occluded architectural pores (geometrical accuracy conditions)
- B) a good strut and nodes connectivity (macro-structural integrity condition) and
- C) a process-related porosity of 0.5% utmost (micro-structural integrity condition).

The three criteria just outlined guided the multi-objective L-PBF process optimisation of this work, and they were used to filter progressively (as a set of sieves) the manufacturing conditions.

The only lattices characterised by $P_A \geq 50\%$ were those fabricated with C2 and C3 settings (whose 3D reconstruction are shown in Figure 34), with a P_A of 50.91% and 64.56%, respectively. The equivalent strut diameters for C2 and C3 manufactured lattices were 285 ± 38 μm and 256 ± 31 μm , respectively, and they represented the smallest strut diameters among the tested conditions. Furthermore, the lattices did not exhibit any occluded porosity P_o . As such, C2 and C3 process settings passed the first selection step for process optimization on the geometrical accuracy (Figure 34).

As previously discussed in this chapter, C2 and C3 conditions were realised for $P=150$ W, the minimum laser power level tested in this study. Lattices fabricated at higher laser powers, 200 W and 250 W, (conditions C4-C9) resulted in excessive material melting, which led to thickened struts and $P_A < 50\%$. Therefore, $P=150$ W represents the maximum laser power for the fabrication of the designed Zr702 octet lattice structures.

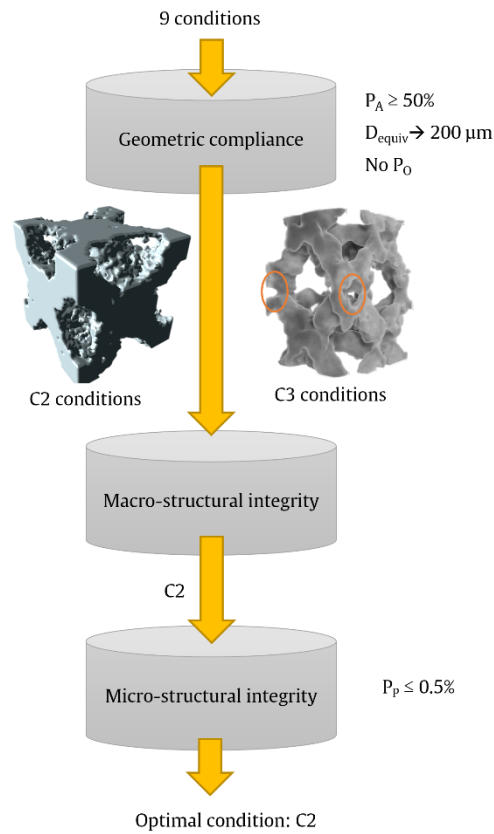


Figure 34 Results of the optimisation of the L-PBF process (island strategy) for the fabrication of Zr702 octet lattices with a unit cell size of 1.1 mm and a nominal strut size of 200 μm .

The lattice produced with C2 was characterised by highly interconnected architectural pores and good strut connectivity (a representative cross section is shown in Figure 30). As such, C2 provided a suitable process setting for the fabrication of macro-structural integer lattices and passed the second criterion for the process optimization. The lattice fabricated with C3, however, was affected by a significant number of broken struts and through holes in the nodes (as previously shown in Figure 33), which might negatively impact the overall lattice mechanical properties. For this reason, C3 was excluded from the list of candidate optimal process settings.

The lattice fabricated with the conditions C2 satisfied also the third criterion on the internal micro-porosity (process defects, P_p), which was below the allowed limit with a $P_p=0.4\%$.

Overall, the process setting C2 ($P=150$ W; $v_s=1400$ mm/s; $h_s=60$ μm) enabled the manufacturing of lattices characterised by P_A of $\sim 51\%$, D_{equiv} of 285 ± 38 μm , and good connectivity of P_A . The lattice satisfied the requirements on the macro-structural integrity, exhibiting good strut and nodes connectivity, and on the microstructural integrity. Therefore, C2 represents an optimal set of laser power, scanning speed and hatch spacing for the production of Zr702 orthopaedic-aimed lattices.

Nevertheless, the geometric mismatch between C2-fabricated lattice and the target design is rather important: the deviation from the designed P_A is $\Delta P_A = -15\%$, whilst $\Delta \Phi S \sim +40\%$. Therefore, the process could be further optimised in order to achieve $P_A \sim 65\%$ whilst maintaining a good macro-structural integrity. As P_A strongly affects the expected mechanical properties of lattice structures, further increasing the P_A to the designed value would mean reducing the elastic gradient of the structure, potentially matching the natural trabecular bones' moduli.

Overall, this work represents the first study on the L-PBF optimisation of Zr702 lattices and should be regarded as the foundation for future work. The DOE used in this study, combined with the X-CT characterisation of the fabricated lattices and the original multi-objective optimisation strategy, allowed to identify a suitable set of the main process parameters (P , v_s , and h_s) for the fabrication of orthopaedic lattices.

The results of the work highlight the challenges encountered in the manufacturing of high-quality orthopaedic lattice-based implants. In particular, it confirmed that it is rather difficult to fabricate octet micro-trusses with high P_A and strut size close to 200 μm , as previously observed in [2-4].

4.4 Fabrication of Zr702 lattices with the contour strategy and automatic dimensional measurement of struts and nodes

Following the first L-PBF optimisation study, a second experimental campaign was launched moving from the optimal set of parameters C2, in the attempt to improve the

geometrical accuracy of the fabricated lattices. The second objective of the study was to characterize the dimensions of the lattice struts and nodes with an automatic method. Here, the contour strategy was selected as it is recommended for the L-PBF manufacturing of lattices, and the scanning speed and the contour distance were varied. In particular, the scanning speed was varied on four levels from 1400 mm/s (the baseline found in the previous experiment) to 1800 mm/s, whilst the contour distance was varied on two levels: 50 μm and 75 μm . The laser power was 150 W, following the C2 baseline.

Table 12 Results of the X-CT characterisation of L-PBF fabricated lattices in contour strategy. ρ_{rel} is the relative density; P_A is the architectural (connected) porosity; P_P is the process-related porosity.

MC	v_s (mm/s)	CD (μm)	ρ_{rel} (%)	P_A (%)	P_P (%)
L1	1400	50	47.01%	52.92%	0.16%
L2	1600		43.11%	56.84%	0.13%
L3	1700		46.61%	53.33%	0.12%
L4	1800		45.21%	54.73%	0.13%
L5	1400	75	43.45%	56.43%	0.26%
L6	1600		41.58%	58.31%	0.25%
L7	1700		42.75%	57.16%	0.23%
L8	1800		42.16%	57.74%	0.22%

The results of the X-CT characterisation of the fabricated lattices are shown in Table 12. Overall, the P_A ranged between ~53-58%. Although a slight improvement from the lattice fabricated with the C2 condition was achieved, switching from the island strategy to the contour one did not lead to significant ameliorations in terms of geometrical accuracy. In terms of micro-structural integrity, though, the contoured lattices exhibited significantly lower P_P (0.16-0.26%) compared to the C2 lattice (0.4%). It was observed that the increase in v_s from 1400 mm/s to 1800 mm/s did not lead to significant variations in the P_A , nor in the P_P . Increasing the contour distance from 50 μm to 75 μm , instead, resulted in a slightly increased P_A and in significantly higher P_P , which passed from an average of ~0.13% to 0.24%.

The original algorithm developed in MATLAB® by the author allowed to automatically measure the diameters of struts and nodes of the octet lattices fabricated with the conditions L1-L8 along the different orientations. In particular, the method allowed to measure the diameter of horizontal and diagonal (oriented at 45° from the building plane) struts, and the dimensions of the nodes in the horizontal and vertical planes.

This method – that entailed the rotation of the 3D matrix of the segmented X-CT data along the directions of interest and the generation of 2D cross-sections that are orthogonal to such directions– represents the first protocol that enables the automatic measurement and characterization of lattice features in more than one direction.

The MATLAB code was validated on the X-CT segmented data relative to the condition C2, for which the dimensions of the struts had been measured manually in ORS Dragonfly on more than fifty struts.

The results of the geometrical characterisation are reported in the tables below: the equivalent diameter (D_{equiv}), the orthogonal diameter (D_{ortho}), the maximum and minimum Feret diameters (MaxFeret and minFeret, respectively), the major and minor elliptical axis (major axis and minor axis, respectively) of the diagonal and horizontal struts are shown in Table 13 and Table 14, respectively.

Table 13 Automatic dimensional characterisation of the diagonal struts relative to the lattices manufactured with the contour strategy (L1-L8) and with the optimal island strategy condition (C2). All the results are expressed by the average value $\pm$ standard deviation.

Diagonal struts relative to lattice	D_{Eqv} (μm)	D_{Ortho} (μm)	MaxFeret (μm)	minFeret (μm)	Major Axis (μm)	Minor Axis (μm)
L1	281 $\pm$ 17	283 $\pm$ 27	369 $\pm$ 56	266 $\pm$ 23	339 $\pm$ 57	252 $\pm$ 23
L2	286 $\pm$ 19	276 $\pm$ 36	400 $\pm$ 75	257 $\pm$ 35	369 $\pm$ 70	245 $\pm$ 33
L3	282 $\pm$ 18	275 $\pm$ 33	397 $\pm$ 78	249 $\pm$ 34	373 $\pm$ 83	239 $\pm$ 31
L4	285 $\pm$ 18	285 $\pm$ 29	389 $\pm$ 86	267 $\pm$ 25	362 $\pm$ 83	251 $\pm$ 27
L5	285 $\pm$ 16	280 $\pm$ 30	382 $\pm$ 88	262 $\pm$ 27	353 $\pm$ 82	254 $\pm$ 27
L6	274 $\pm$ 16	277 $\pm$ 26	348 $\pm$ 80	262 $\pm$ 22	321 $\pm$ 69	253 $\pm$ 21
L7	280 $\pm$ 16	279 $\pm$ 35	370 $\pm$ 81	262 $\pm$ 31	344 $\pm$ 83	251 $\pm$ 30
L8	280 $\pm$ 18	280 $\pm$ 31	363 $\pm$ 69	262 $\pm$ 29	337 $\pm$ 69	252 $\pm$ 26
C2	287 $\pm$ 19	268 $\pm$ 48	416 $\pm$ 121	251 $\pm$ 44	390 $\pm$ 123	244 $\pm$ 44

Table 14 Automatic dimensional characterisation of the horizontal struts relative to the lattices manufactured with the contour strategy (L1-L8) and with the optimal island strategy condition (C2). All the results are expressed by the average value $\pm$ standard deviation.

Horizontal struts relative to lattice	D _{Equiv} (μm)	D _{Ortho} (μm)	MaxFeret Diameter (μm)	minFeret Diameter (μm)	Major Axis (μm)	Minor Axis (μm)
L1	280 $\pm$ 18	278 $\pm$ 31	381 $\pm$ 51	250 $\pm$ 30	366 $\pm$ 57	236 $\pm$ 26
L2	273 $\pm$ 14	282 $\pm$ 28	357 $\pm$ 35	260 $\pm$ 22	333 $\pm$ 38	246 $\pm$ 23
L3	272 $\pm$ 15	282 $\pm$ 46	535 $\pm$ 1335	257 $\pm$ 39	538 $\pm$ 1552	244 $\pm$ 38
L4	273 $\pm$ 17	262 $\pm$ 70	958 $\pm$ 2416	241 $\pm$ 63	1036 $\pm$ 2807	225 $\pm$ 59
L5	272 $\pm$ 18	268 $\pm$ 45	533 $\pm$ 1265	248 $\pm$ 40	549 $\pm$ 1502	230 $\pm$ 38
L6	286 $\pm$ 18	274 $\pm$ 30	392 $\pm$ 447	255 $\pm$ 25	377 $\pm$ 518	244 $\pm$ 22
L7	269 $\pm$ 14	279 $\pm$ 30	355 $\pm$ 40	255 $\pm$ 20	335 $\pm$ 47	239 $\pm$ 20
L8	272 $\pm$ 20	270 $\pm$ 34	431 $\pm$ 754	248 $\pm$ 26	424 $\pm$ 877	232 $\pm$ 28
C2	287 $\pm$ 18	288 $\pm$ 33	359 $\pm$ 60	271 $\pm$ 29	341 $\pm$ 64	262 $\pm$ 28

The data in Table 13 and Table 14 show that all the lattices were characterized by struts thicker than 200 μm , as expected from the measured P_A (always lower than the nominal value, 65%). Moreover, no significant difference was recorded between the diagonal struts of the lattices fabricated with L1-L8 and the baseline condition C2 (Table 13), nor between the horizontal struts fabricated with the same conditions (Table 14).

A representative series of diameters for the diagonal struts is visualised in Figure 35a, which demonstrates the equivalent diameter is substantially equal to the orthogonal diameter, whilst the maximum Feret diameter and the major axis are significantly higher than the equivalent diameter, the minimum Feret diameter and the minor elliptical axis. These results indicate that the strut shape of the diagonal strut is elliptical, rather than circular, as it can be appreciated from the X-CT image reported in Figure 35a.

The same conclusion was drawn for the horizontal struts of the lattices fabricated with the conditions L1, L2, L7 and C2. In the case of the lattices manufactured with the conditions L3-L6 and L8, instead, the extreme values of standard deviation recorded for the maximum Feret diameter and the major axis (highlighted in red Table 14) indicate a non-normal distribution of the maximum diameters of the measured struts, and suggest significant deviations from the circularity and irregular strut shapes. The visual analysis of the X-CT lattice cross-section confirmed the irregularity and variety of shapes of the horizontal

struts, which can be appreciated in Figure 35c. Such phenomenon could be explained by considering the generation of the horizontal struts in the L-PBF process; the material in the powder bed is melted on a heterogeneous layer: some powder is welded on the nodes underneath, whilst some other (the central portion of the strut) is generated as an overhanging feature over other layers of metallic powder (refer to Figure 4 in Chapter 2). The inner heterogeneity experienced by the metallic powder during these stages of the manufacturing process may be the origin of the irregularity of such struts in the octet lattice, but further study is needed to confirm this hypothesis.

The diameters of the diagonal struts with the horizontal struts were compared to investigate the potential effect of the strut orientation with respect to the building platform on the geometrical accuracy. Figure 35b shows the stability of the equivalent diameter, regardless of the strut orientation. It was concluded that the building orientation does not have an effect on the geometrical accuracy of the struts in the L-PBF manufacturing, indicating the manufacturing repeatability of the L-PBF process for the fabrication of lattice structures in this study [176].

The results relative to the nodes measured on the XY plane and XZ plane are reported in Table 15 and Table 16, respectively.

Table 15 Automatic dimensional characterisation of the nodes relative to the lattices manufactured with the contour strategy (L1-L8) and with the optimal island strategy condition (C2), measured on the XY plane. All the results are expressed by the average value $\pm$ standard deviation.

Nodes measured on the XY plane relative to lattice	D _{Equiv} (μm)	D _{Ortho} (μm)	MaxFeret Diameter (μm)	minFeret Diameter (μm)
L1	571 $\pm$ 24	675 $\pm$ 67	767 $\pm$ 81	581 $\pm$ 42
L2	563 $\pm$ 28	666 $\pm$ 75	763 $\pm$ 85	580 $\pm$ 50
L3	570 $\pm$ 29	662 $\pm$ 86	805 $\pm$ 83	585 $\pm$ 55
L4	570 $\pm$ 30	669 $\pm$ 82	795 $\pm$ 81	589 $\pm$ 50
L5	560 $\pm$ 30	663 $\pm$ 87	760 $\pm$ 85	573 $\pm$ 52
L6	561 $\pm$ 32	683 $\pm$ 89	781 $\pm$ 95	582 $\pm$ 57
L7	562 $\pm$ 28	663 $\pm$ 87	788 $\pm$ 82	581 $\pm$ 52
L8	562 $\pm$ 29	669 $\pm$ 86	792 $\pm$ 82	586 $\pm$ 53
C2	561 $\pm$ 34	594 $\pm$ 84	760 $\pm$ 102	538 $\pm$ 68

Table 16 Automatic dimensional characterisation of the nodes relative to the lattices manufactured with the contour strategy (L1-L8) and with the optimal island strategy condition (C2), measured on the XZ plane. All the results are expressed by the average value $\pm$ standard deviation.

Nodes measured on the XZ plane relative to lattice	D _{Equiv} (μm)	D _{Ortho} (μm)	MaxFeret Diameter (μm)	minFeret Diameter (μm)
L1	586 $\pm$ 48	690 $\pm$ 100	825 $\pm$ 104	614 $\pm$ 73
L2	574 $\pm$ 66	607 $\pm$ 128	835 $\pm$ 145	544 $\pm$ 110
L3	579 $\pm$ 66	596 $\pm$ 140	850 $\pm$ 131	539 $\pm$ 120
L4	575 $\pm$ 61	604 $\pm$ 134	965 $\pm$ 1077	543 $\pm$ 105
L5	623 $\pm$ 40	724 $\pm$ 87	863 $\pm$ 267	616 $\pm$ 74
L6	544 $\pm$ 37	614 $\pm$ 89	788 $\pm$ 303	553 $\pm$ 65
L7	615 $\pm$ 42	703 $\pm$ 106	991 $\pm$ 1140	606 $\pm$ 88
L8	617 $\pm$ 41	711 $\pm$ 87	868 $\pm$ 100	613 $\pm$ 74
C2	555 $\pm$ 62	585 $\pm$ 114	814 $\pm$ 124	525 $\pm$ 93

The nodes were characterized by equivalent diameters in the range of 555-615 μm , on average (Table 15 and Table 16).

The measurements performed on the XY plane (Table 15) for the D_{equiv}, D_{Ortho}, maximum and minimum Feret diameters were in the same range in all the manufactured lattice. The general trend for these diameters is shown in Figure 35d, which demonstrates that the maximum Feret diameter is significantly higher than D_{equiv} and the minimum Feret diameter.

No significant difference was found between the measurements performed on the plane XY and the plane XZ, as illustrated in Figure 35e, confirming the manufacturing repeatability of the L-PBF machine used in this study.

In summary, the lattices fabricated with the contour strategy were characterized by slightly higher P_A than C2, with a maximum increase of 7% in P_A, but their struts and nodes did not show any significant differences with C2. Nevertheless, the fabrication with contour strategy led to significantly improved micro-structural integrity with respect to C2.

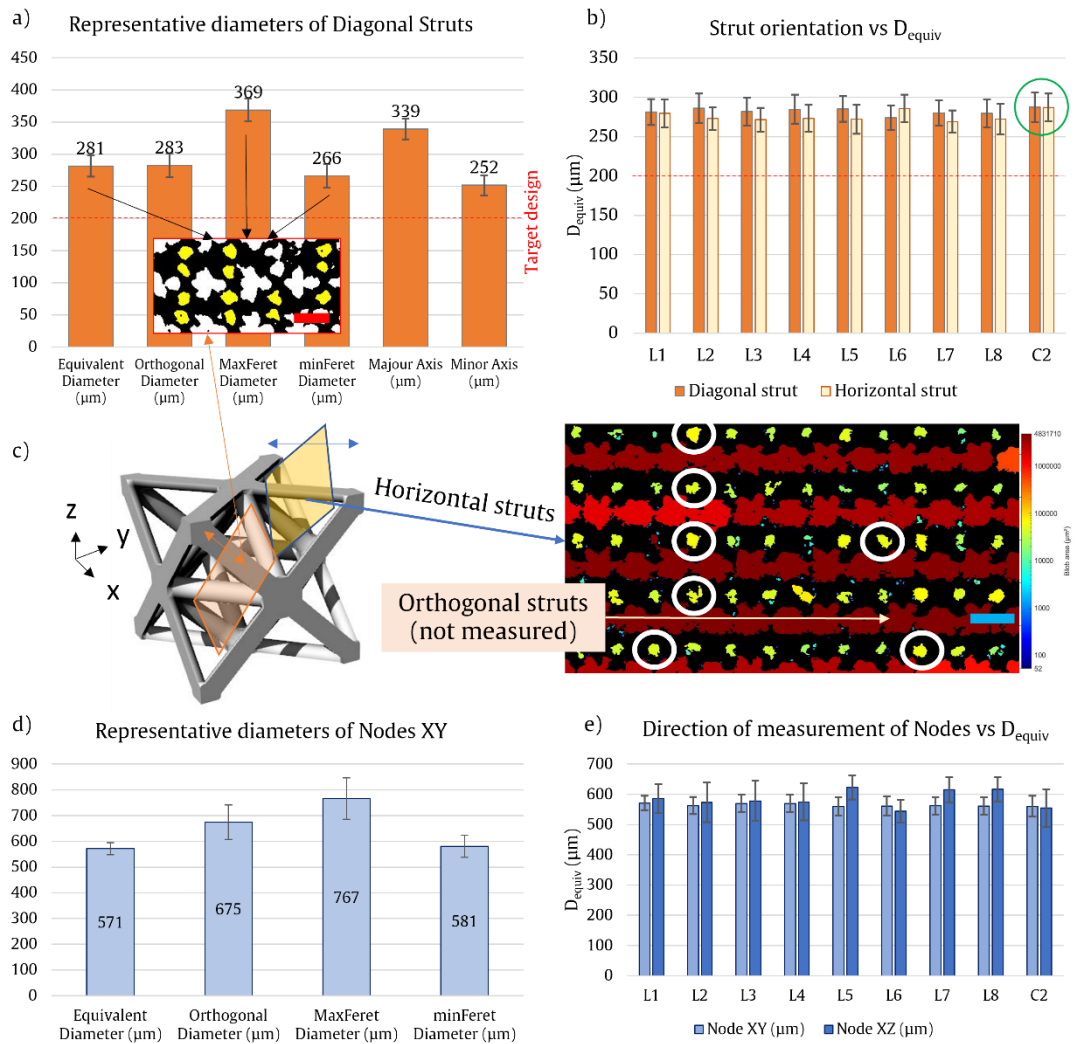


Figure 35 a) A representative series of diameters relative to the struts built at 45° from the building platform (diagonal strut), and a cross-section of the lattice highlighting (in yellow) the struts being measured by the automatic image analysis (scale bar is 1 mm). The series is relative to the lattice fabricated with L1 settings; b) The equivalent diameter D_{equiv} of the diagonal and horizontal struts for the L1-L8 and C2 fabricated lattices; c) 3D model of the octet lattice and a representative colour-coded cross-section of X-CT image showing the horizontal (measured) struts and the orthogonal horizontal (not measured) struts. Scale bar is 1mm. d) A representative series of diameters relative to the nodes measured on the XY plane; the series is relative to the lattice fabricated with L1 settings; e) The equivalent diameter D_{equiv} of the nodes measured on the XY and XZ planes for the L1-L8 and C2 fabricated lattices.

4.5 Conclusions and recommendations

The studies on the optimisation of the L-PBF process parameters for the fabrication of the Zr702 octet lattice structures here performed were motivated by the lack of published knowledge on the processing of Zr702 powders for thin-walled structures.

This represents the first work aimed at printing Zr702 lattice structures and contributes to the knowledge of the L-PBF processing for such material. In particular, the research allowed to draft the process diagrams for the process-related porosity (P_p) and for the occluded porosity (P_o), and to understand how the tested process parameters affected the geometrical accuracy, the macro-structural and micro-structural integrity of the L-PBF fabricated lattices.

It was concluded that the main process parameters for the geometrical accuracy are, in order, the laser power and the scanning speed. The hatch spacing (in the island strategy) and the contour distance (in the contour strategy) did not play a significant role in the tested levels. The micro-structural integrity of the lattice was significantly affected by the combined effects of P and v_s . However, the energy density E_d (J/mm^2) -which directly depends on P and v_s - did not show strong correlation with the micro-structural defects (P_p). The assessment of the macro-structural integrity – which can be evaluated only qualitatively –resulted critical for the complete understanding of L-PBF process for the lattice fabrication, enabling the correct identification of the “balling” process conditions in the process diagram for P_p (which is associated with the micro-structural integrity). Therefore, the author recommends characterizing both the macro-and the micro-structural integrity during the optimisation process studies.

The optimisation strategy developed by the author led to the identification of a set of parameters ($P=150$ W; $v_s=1400$ mm/s and $h_s=60$ μ m) to be used with the “island strategy” to manufacture suitable Zr702 octet lattices for an orthopaedic application, which were characterised by a P_A of 51%, a good material connectivity (macro-structural integrity) and a P_p of ~0.4%. This structure was selected for further testing: its mechanical performance

is presented and discussed in Chapter 5, whilst its behaviour in the biological tests is presented in Chapter 6.

The lattices fabricated with the “contour strategy” were all characterised by a $P_A > 50\%$, a good macro-structural integrity and a $P_P < 0.5\%$, and therefore are considered suitable for an orthopaedic application according to the selected criteria of this study. In fact, the micro-structural integrity was significantly improved (with respect to the optimal island strategy condition) with the introduction of the contour, and the contour distance $CD=50\text{ }\mu\text{m}$ led to the lowest P_P (0.12-0.16%). Nevertheless, the contour strategy did not lead to a significant improvement in the geometric accuracy of the lattice with respect to the optimal island strategy conditions.

The author believes that the geometrical accuracy of the Zr702 octet lattices might be significantly improved by reducing the laser power to 100 W and recommends to further investigate the effect of the printing strategy in the generation of the lattice struts and nodes on different orientations.

The algorithm developed in this study allows to fully characterise the geometry of the octet lattices automatically and might be used for the assessment of Zr702 octet lattices in new optimisation studies. The algorithm can be used to characterize the lattice topologies that exhibit horizontal and 45° -oriented struts, such as centred-body cubes, Kelvin structures, etc. Moreover, the MATLAB codes can be easily adapted to extract 2D images with the lattice cross-sections in other directions.

Chapter 5

Mechanical behaviour of L-PBF Zr702 and Zr702 lattice: Results and Discussion

In this chapter, the results relative to the mechanical tests performed on the L-PBF manufactured Zr702 (bulk) cylinders and lattices manufactured with the process condition C2 are presented and discussed.

The objective of the tests was to assess the mechanical behaviour of both the material and the lattice structure made in Zr702, in order to assess the suitability of the fabricated lattices for load-bearing orthopaedic implants from a mechanical perspective.

As mentioned already in Chapter 2, the lattice mechanical behaviour is not an intrinsic property, but depends both on the material used and the lattice structure topology.

The chapter will firstly present the results relative to the material (Zr702). Then, it will illustrate and discuss the experimental mechanical behaviour of the lattice, with particular reference to its elastic gradient and compressive proof strength. As previously described (Chapter 2), these features are critical in the orthopaedic application. Finally, it will demonstrate the mechanical suitability of the lattice for orthopaedic implants.

5.1 Mechanical behaviour of L-PBF Zr702 material under compression

In this section, the results relative to the mechanical testing of Zr702 bulk cylinder are presented, and the Elastic modulus of the L-PBF Zr702 material is calculated.

The compression test performed with Digital Image Correlation (DIC) analysis and in cyclic loading conditions allowed the accurate measurement of the sample lengths and incremental length variations at specific time intervals and throughout the test. This

allowed to calculate accurately the true strains and engineering strains, and finally to calculate correctly the L-PBF Zr702 (compressive) elastic modulus.

The section is divided into two parts: the first illustrates the derivation of the engineering stress vs engineering strain curve of the full compression test; the second shows the elastic modulus of the L-PBF Zr702 and discusses the results with the literature.

5.1.1 The engineering stress-strain compression curve of L-PBF Zr702

The engineering stress vs engineering strain curve for the L-PBF Zr702 bulk cylinder was derived in MATLAB by using the in-house script “CompressionTestDataAnalysis.m” (which can be found in Appendix I). Briefly, the True Strains were calculated from the Instant length (mm) and Instant Length variations (mm) – that had been measured by DIC. Because several outliers were noticed (see Figure 36a, blue plot), the true strains were subjected to a denoising operation, that removed and substituted the outliers (orange curve in Figure 36a). The engineering strains were hence calculated from the denoised true strains (Figure 36b). This intermediate step (the calculation of true strains before the engineering strains) was necessary because the denoising operation was not satisfactory when it was performed on the engineering strains calculated directly from the instant lengths (data not shown).

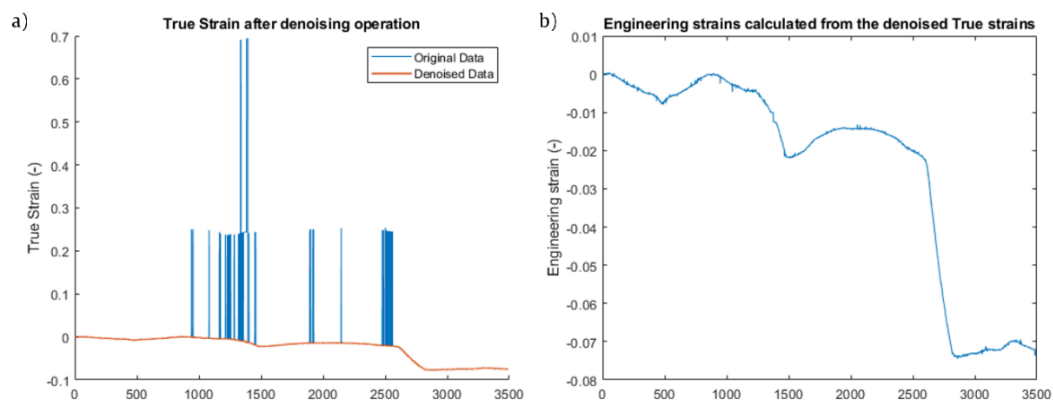


Figure 36 a) True strains calculated from the values measured by DIC (Digital Image Correlation) – original data, in blue – and the Denoised True Strain (orange line): the denoising operation was performed in MATLAB by removing the outliers and filling the missing values with the nearest mean value of strains; b) the Engineering strains, that were calculated from the denoised True strain values.

To determine the engineering stress-strain curve, the data relative to the engineering strain derived from the DIC analysis (Figure 37b) had to be coupled with the data relative to the load recorded by the compression machine (Figure 37a).

As it can be noted from Figure 37, the maxima and minima points (highlighted in orange) define the time ranges for each step (loading, unloading) of the cyclic loading test. Because the frequency of data recording differs from the compression machine to the DIC analysis and in particular is higher for the load data, the engineering strain vs time from DIC was interpolated – for each loading/unloading step – to match the frequency of the load data. The reader is referred to Appendix I for the detailed coupling procedure.

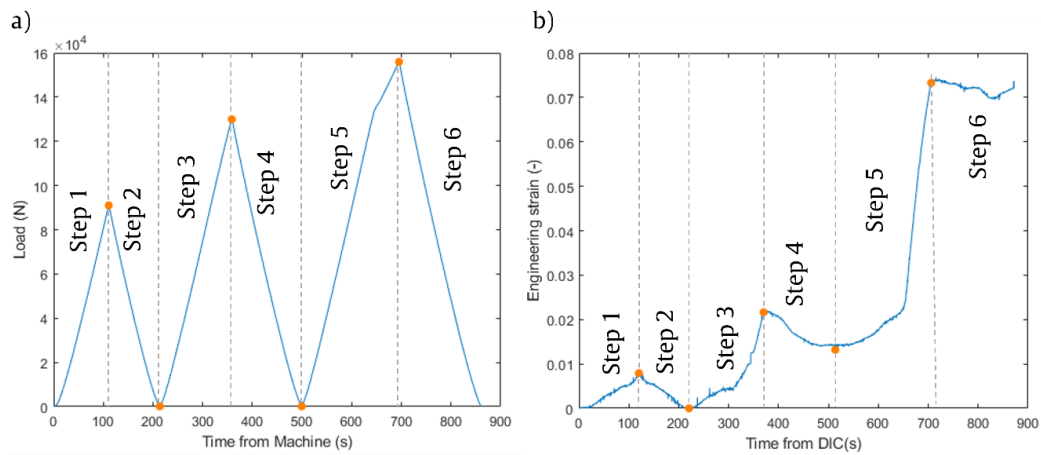


Figure 37 Evolution of load (a) and engineering strain (b) over the time from the machine and the time from Digital Image Correlation (DIC) analysis, respectively. The maxima and minima points from the loads over the machine (a) and from the absolute engineering strains over the DIC time (b) are highlighted in orange. Such maxima and minima define the six steps of loading-unloading cycles performed in the compression test, and were used to couple accurately the stress and strains in the engineering stress – engineering strain curve, reported below.

The Engineering Stress – Engineering Strain curve relative to the L-PBF Zr702 is reported in Figure 38. Engineering stresses increase linearly with the engineering strains until ~450 MPa, that is the proportionality limit. Beyond 450 MPa, stresses deviate from linearity to form a long knee between 600-1000 MPa. The yielding point (here determined graphically) is similar to the yield strength (425-431 MPa) of bulk Zr702 manufactured along the same direction by L-PBF and tested in tension by Aristizabal *et al.* [7].

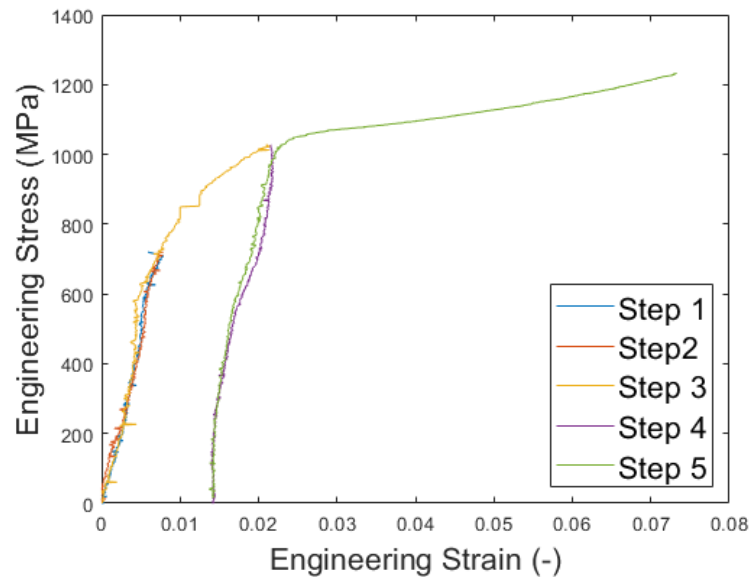


Figure 38 Engineering Stress - Engineering Strain curve for the L-PBF Zr702 bulk cylinder. Engineering strains were derived from the Digital Image Correlation (DIC) analysis.

Two hysteresis loops can be spotted in the curve. The first (realised between Step 1 and Step 3, for stresses below 720 MPa) covers the elastic region and the incipient yielding; the second occurs beyond the yielding point (Step 4). The portions relative to the unloading (Step 2 and Step 4 in Figure 38) should follow a linear trend. Nevertheless, this was not observed in Step 4, in which important deviations from linearity are observed.

The maximum flow stress (measured before the final unloading) was 1233 MPa (or 1.2 GPa).

5.1.2 Elastic modulus of L-PBF Zirconium

The estimation of the elastic modulus of bulk Zr702 was performed from the engineering stress-engineering strain curve presented above (Figure 38).

In principle, the elastic modulus could be estimated from the two unloading traits (Step 2 and Step 4, in Figure 38). However, given the significant deviations from linearity observed in the second unloading (Step 4, Figure 38), the elastic modulus of the L-PBF manufactured Zr702 was estimated only from the portion of the Engineering Stress – Engineering Strain curve relative to the first unloading (Step 2, Figure 38).

The elastic modulus was calculated with the secant method, by using the minimum stress recorded in Step 2 and 400 MPa (below the proportionality limit, as strict tolerance on linearity was applied) and the relative strains. The compressive elastic modulus was 94.75 GPa, as shown in Figure 39. The so-estimated modulus is a chord modulus following the ASTM 111-27 standard [177]. Nevertheless, the modulus here derived can be compared also with Young's moduli as the upper stress-strain coordinate was in the linear elastic range (i.e., the stress was below the proportionality limit).

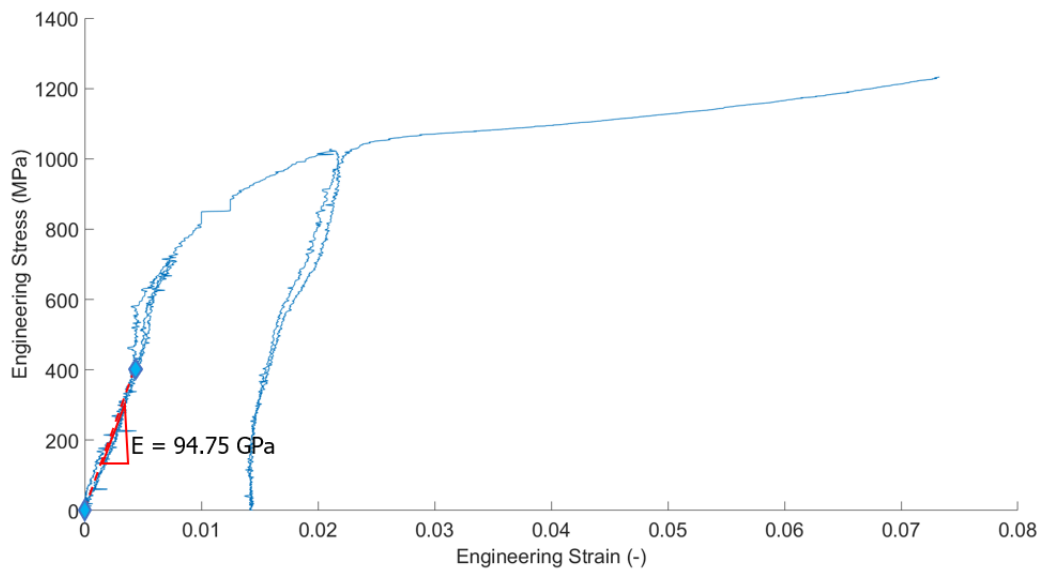


Figure 39 The elastic modulus of Zirconium (Zr702) estimated from the linear portion of the unloading trait in the first cycle (Step 2) in the Engineering Stress vs Engineering Strain curve by secant method. The blue diamonds indicate graphically the stress-strain coordinates used to calculated the elastic modulus (E).

The compressive modulus is higher than the tensile Young's modulus ($87.6 \div 90$ GPa) of Zr702 manufactured by the same L-PBF machine at the same manufacturing conditions (process parameters and sample building direction) obtained by Aristizabal *et al.* [7]. Nonetheless, the compressive elastic modulus of Zr702 obtained in this study is lower than the wrought material's elastic modulus reported in the literature 99.2 GPa [65, 178].

5.1.3 Considerations on the method developed for the material testing

Early compression tests had been performed on Zr702 cylinders with poor results (data not shown). Testing the material under compression posed several challenges. Among the

others, the restriction on using extensometers during these tests impaired the accurate estimation of the deformations in the elastic regime.

Nevertheless, the introduction of DIC analysis to the compression test allowed to track the displacements of single points in the speckle pattern at small deformations, and thus to calculate accurately the true strains in the material during its compression, including in the elastic regime. Moreover, testing the material under cyclic loadings allowed to recover the elastic deformations in the unloading steps and to determine the linear elastic regime. As demonstrated earlier, data denoising of DIC-derived true strains was essential to couple accurate deformations with stresses, and to obtain the elastic modulus of the material.

As a further improvement of the material testing in compression, the author would recommend performing tests on taller samples, as this would allow to monitor a larger speckle (hence the displacements of more points) during the DIC analysis. Indeed, increasing the number of speckle points (hence displacements at each frame) would result in reduced noise in the output data (true strains).

5.2 Mechanical behaviour of Zr702 lattice structures

In this section, the results of the mechanical testing for the Zr702 lattices manufactured with the process settings C2 are presented and discussed.

First, the results relative to the loading-only compression curves (relative to the Preliminary test according to ISO 13314 [93]) are reported and analysed, with particular reference to the Plateau Stresses (the key objective of this mechanical test) and the Quasi-elastic Gradient of the lattices.

Second, the mechanical behaviour of the lattice in cyclic loading (according to ISO 13314 [93]) is illustrated, and strong emphasis is given to the Elastic Gradient for the lattice structure, the ultimate objective of the mechanical testing for this study.

5.2.1 Preliminary test ISO 13314: Stress-Strain compression curves

Three cube-like (edge = 11 mm) zirconium lattice samples were tested in the ISO 13314 preliminary test [93].

The specimen “MT-0319 Sp3 R” was compressed until an engineering strain of 0.26, due to severe shearing of the sample. Samples “MT-0319 Sp4 R” and “MT-0319 Sp5 R” were compressed until 0.63 and 0.65, respectively.

The Stress-Strain compression curves of the preliminary are reported in Figure 40. The curves relative to “MT-0319 Sp4 R” and “MT-0319 Sp5 R” exhibit the three typical zones of porous structures under compression: the elastic region (I), followed by a relatively long plateau (II), and the densification region (III).

The compression curves overlap each other, suggesting a very good repeatability of the mechanical behaviour of lattices under compressive loading conditions. This finding also suggests that the L-PBF manufacturing with C2 settings is robust and enables the fabrication of lattices with a reliable mechanical behaviour.

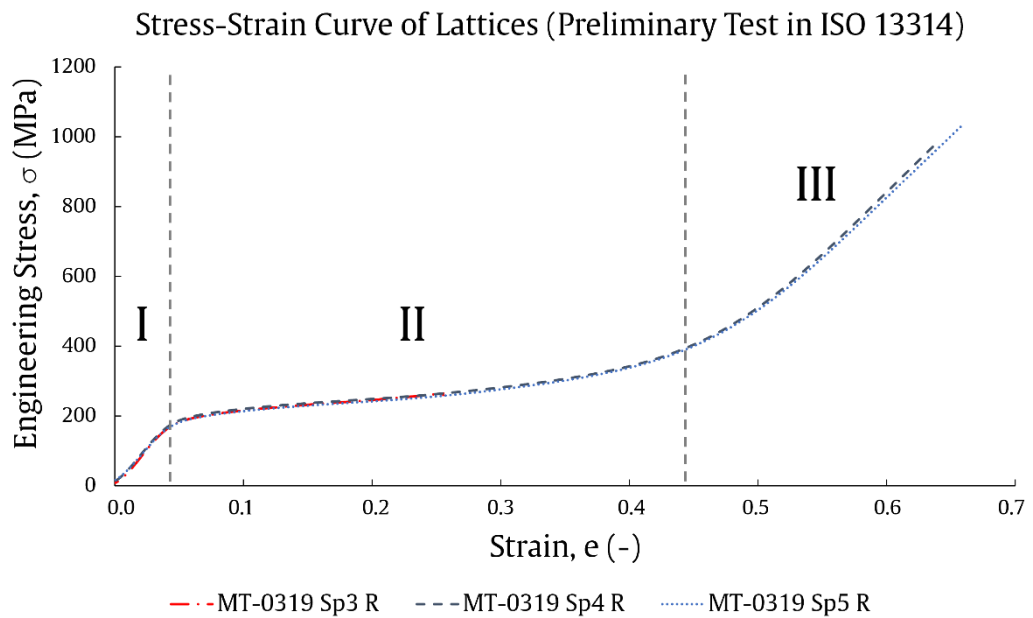


Figure 40 Stress-Strain Curves of zirconium lattices relative to the preliminary test of ISO 13314 for the estimation of the plateau stresses. Areas I, II, and III in the graph identify the elastic region, plateau-like region and the densification region, respectively.

The plateau stress was evaluated between 0.2 and 0.3 of (engineering) strain, on the curves of “MT-0319 Sp4 R” and “MT-0319 Sp5 R”, according to the ISO 13314 [93]. “MT-0319 Sp3 R” was excluded from this analysis due to the interruption of the curve at 0.26 strain. Table 17 reports the Plateau stress, the 70% and the 20 % of the plateau stress (called $\sigma_{70\%}$ and $\sigma_{20\%}$, respectively). These latter were used to determine the loads for the unloading test, which are hereafter called $F_{70\%}$ and $F_{20\%}$. The loads were calculated using the effective sample area (A). Finally, the maximum values for the $F_{70\%}$ and $F_{20\%}$ loads (23 kN and 6.6 kN, respectively) were selected to carry out the following unloading test.

Table 17 Results of the Preliminary test of ISO 13314.

Sample name	σ_{Plateau} (MPa)	$\sigma_{70\%}$ (MPa)	$\sigma_{20\%}$ (MPa)	A (mm ²)	$F_{70\%}$ (kN)	$F_{20\%}$ (kN)
MT-0319 Sp4 R	260	182	52	127	23	6.6
MT-0319 Sp5 R	250	175	50	127	22	6.3

5.2.2 The particular behaviour of the plateau stage in the stress-strain curve

The evolution of the stresses for increasing strains observed in stage II of the stress vs strain curve does not follow the typical behaviour of the stretch-bending dominated structures, to which the octet belongs (in principle). In fact, the small (but steady) increase of the stresses for large increases in strain recalls the relatively flat plateau region of the bending-dominated structures.

The same observation was made on other AM lattice structures investigated in the literature. Benedetti *et al.* [86] explains this shift in behaviour (from stretching to bending dominated) with the geometric inaccuracies introduced by the AM process into the fabricated lattices. They suggested that strut misalignment with respect to the loading direction might cause bending effects. In addition to this, however, the authors highlighted the limitations of the Gibson-Ashby theory, that loses accuracy when the relative density is greater than 0.3, as the lattice struts are assumed to be “sufficiently slender”. Moreover,

the theory is unable to take into account the effect of the stress concentrators, such as the lattice nodes, on the stress-strain curve.

In the case of the Zirconium lattice investigated here, all the factors discussed above might have played a role in the mechanical behaviour of the structure.

However, the author would like to suggest another possible factor to those already mentioned. The octet (ideal) topology is classed as stretch-dominated structure because the Maxwell metric M from the Maxwell's criterion equation is zero (Equation 15). This means that only a variation of one unit more in the nodes (joints, j) or one unit less in the struts (b) would lead to a negative metric M .

Equation 15

$$b - 3j + 6 = M = 36 - 42 + 6 = 0$$

This observation suggests that if the material continuity in one or more struts in the manufactured lattice is interrupted (or, in other words, if the "macro-integrity" of the lattice is lost due to broken struts), the lattice behaves as a metallic foam or a bending-dominated structure, rather than a stiffer and highly efficient stretch-dominated structure.

Nevertheless, the shift in the behaviour from stretch-dominated to bending-dominated structure is not problematic for an orthopaedic application. In fact, for metallic orthopaedic implants (and permanent implants, in general), it is desirable to have metallic structures that are compliant, rather than collapsing by brittle behaviour.

5.2.3 Quasi-Elastic Gradient

The quasi-elastic gradient was calculated by linear regression in the linear elastic region of the stress-strain curves of the preliminary test, in the stress range between 40-130 MPa. The R^2 of all the fitted equations was 0.9999.

The average quasi-elastic gradient of the Zr702 lattice structure was 4.4 ± 0.2 GPa. The values relative to each sample are reported in Table 18.

Table 18 Quasi-elastic gradients of lattices tested in the Preliminary test ISO 13314

Sample name	Quasi-elastic gradient (GPa)
MT-0319 Sp3 R	4.5
MT-0319 Sp4 R	4.4
MT-0319 Sp5 R	4.2
Average $\pm$ SD	4.4 ± 0.2

The average quasi-elastic gradient of the lattices is the 4.6% of the Zr702 (bulk) elastic modulus. Therefore, the porosity of 51% has reduced the material elastic modulus by 95.4%.

As mentioned in Chapter 2, the quasi-elastic gradient does not represent (strictly-speaking) the mechanical stiffness of porous metallic structures. Nevertheless, it is often used for this purpose in the literature as witnessed by the review of Benedetti *et al.* [86]. Therefore, the coordinate E/E_0 and the relative density (0.49, as estimated by X-CT image analysis) were plotted into the diagram originally developed by Benedetti *et al.* [86] and are reported in Figure 41, to allow the comparison between the experimental results obtained in this study and the literature that deals with the mechanical behaviour of lattice structures.

Before proceeding to the comparison, it is worth noting that the reduction in the material's Young's modulus should depend solely on the lattice topology and relative density, whereas it is not affected by the material. Nevertheless, the colour-coding in the figure enables the reader to calculate the effective mechanical properties of the lattice structures fabricated with different materials. Therefore, to allow better graphical result and figure usability, the author colour-coded the Zr702 material in fuchsia.

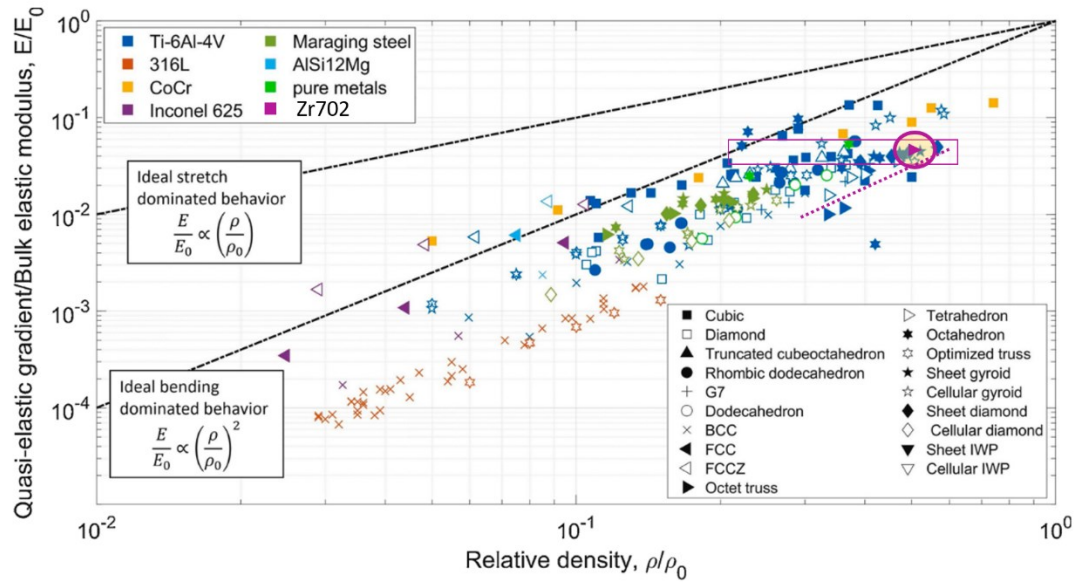


Figure 41 Comparison of the relative elastic modulus vs relative density of the Zr702 lattice manufactured in this study with the properties of various porous metallic structures available in the literature. The fuchsia forward-looking filled triangle is relative to the lattice structure fabricated and investigated in this study. The rectangle highlights the level of reduction of elastic modulus obtained with the Zr702 octet lattice, showing that this can be achieved with different topologies only by significantly reducing the lattice relative densities. The dotted fuchsia line that unites the coordinates relative to octet lattices with different relative densities follows the bending-dominated curve. Image adapted from Benedetti et al. [86].

As highlighted in the rectangle of Figure 41, the Zr702 octet lattice structure fabricated by L-PBF with the C2 conditions led to a modulus reduction that is equivalent to those reported for cubic and octahedron lattices with lower relative densities (down to 0.1) made in Ti6Al4V. This suggests that the octet lattice allows to obtain interesting mechanical properties even at relatively low porosity (50% is deemed to be the minimum porosity for orthopaedic applications), whilst other well-known topologies like cubic or octahedron cells require higher porosity (80% to 90%) to achieve the same stiffness. This has two implications: first, the fact that the same stiffness can be obtained at a lower porosity for the octet lattice means that the yield strength of the structure will be higher, and therefore will have higher load-bearing capacity than the topologies that need 80-90% to have the same effective stiffness. Second, the tolerance to the geometrical errors in the manufacturing of octet lattices for orthopaedic implants can be higher with respect to

cubic or octahedron lattices, where the achievement of the designed architectural porosity becomes critical to obtain porous orthopaedic implants with suitable stiffness.

The fuchsia dotted line in Figure 41 unites the experimental coordinates E/E_0 and relative density of octet lattices. As it can be seen, the line is parallel to the bending-dominated curve, confirming the mechanical behaviour of this topology when the relative density exceeds 0.3.

5.2.4 Unloading test (ISO 13314) and Elastic Gradient of lattice structure

The unloading test was performed on five samples according to the ISO 13314 [93], and the elastic gradient was calculated from the relative stress-strain curves, which are shown in Figure 42.

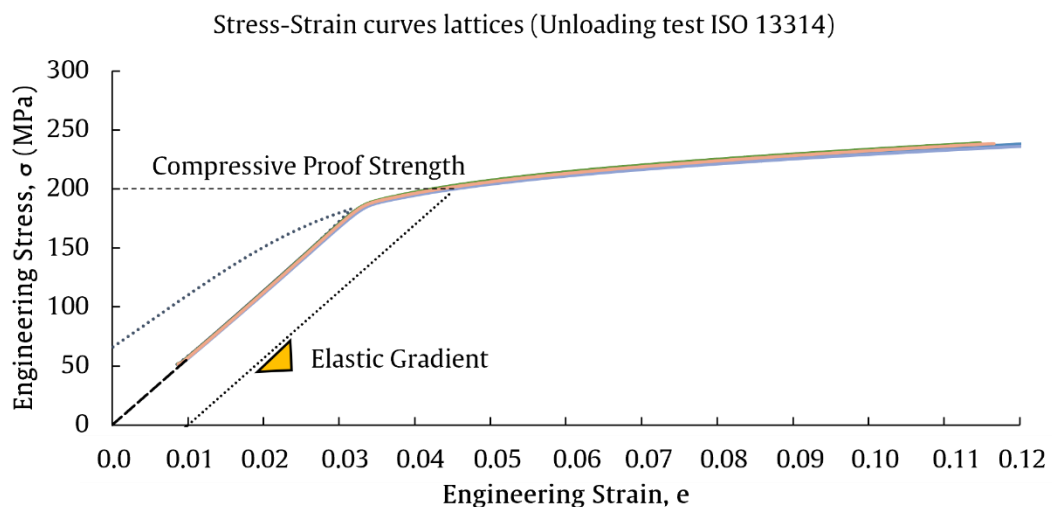


Figure 42 Stress-Strain curves of Zr702 lattices obtained from the compression tests performed according to the unloading test as per ISO 13314. The curves were shifted to match the zero plastic strain, according to the ISO 13314. The compressive proof strength determined at 1% of plastic strain as per ISO 13314 is graphically shown.

The lattices exhibited very similar compression stress-strain curves, confirming that the Zr702 octet lattices fabricated by L-PBF with C2 conditions exhibited a consistent mechanical behaviour, as observed in the preliminary test.

The elastic gradient of the Zr702 octet lattice fabricated with the conditions C2 was calculated according to the ISO 13314 [93] and it resulted equal to 5.64 ± 0.03 GPa. The architectural porosity of 51% led to a reduction of ~94% of the material's Young's

modulus. The compressive proof strength – calculated following the norm [93] - was 201.94 ± 1.23 MPa. The data relative to the tested samples are reported in Table 19.

The average elastic gradient falls within the range of the compact bone's elastic moduli (3-40 GPa [10, 179]), and is close to the lower limit of that range. The lattice's compressive proof strength matches the typical yield strengths of compact bones (100-400 MPa [10, 180, 181]). Hence, the Zr702 octet lattices fabricated with C2 settings match the compact bones mechanical properties and are able to withstand the physiological loads and are fit for use in load-bearing orthopaedic implants [10]. Therefore, the Zr702 octet lattices fabricated in this study appear to be suitable for an orthopaedic application.

Table 19 Elastic gradient (GPa) and compressive proof strength (MPa) of Zr702 octet lattice structure fabricated with conditions C2.

Sample name	Elastic Gradient (GPa)	Compressive Proof Strength (MPa)
MT-0319 Sp1C	5.66	200.36
MT-0319 Sp2C	5.66	203.65
MT-0319 Sp3C	5.57	200.71
MT-0319 Sp4C	5.64	202.35
MT-0319 Sp5C	5.65	202.62

By comparing the obtained elastic gradient with the elastic moduli of various bones, it is possible to suggest a specific orthopaedic application for the fabricated structure. The average elastic gradient of 5.64 GPa makes this Zr702 octet lattice particularly suitable for the substitution of tibia, whose cortical elastic modulus is 5.72 ± 1.27 GPa [182].

Figure 43 illustrates the comparison between the mechanical properties of the Zr702 octet lattice here investigated and the Ti6Al4V octet lattices characterised by various porosities fabricated by Melançon *et al.* [10], which inspired the choice of the octet lattice in this study.

As it can be noted from Figure 43, the Zr702 octet lattices exhibited a higher yield strength (or, more appropriately, compressive proof strength) compared to all the Ti6Al4V octet lattices tested by Melançon *et al.* [10]. Interestingly, this was observed even for Ti6Al4V lattices characterised by porosities lower than the Zr702 octet one (48-49% vs 51%).

Overall, this demonstrates the superior yield strength of Zr702 over Ti6Al4V, and the highest load-bearing capacity of this material over the classic, gold-standard, orthopaedic material.

It is worth noting that the Ti6Al4V octet lattice with a porosity of 66% in [10] matched the strongest and stiffest *trabecular bones* (grey area in Figure 43). Zr702 octet lattice structures with similar porosities might exhibit mechanical properties that match less stiff trabecular bones, potentially allowing to cover the full range of bones moduli (compact and trabecular).

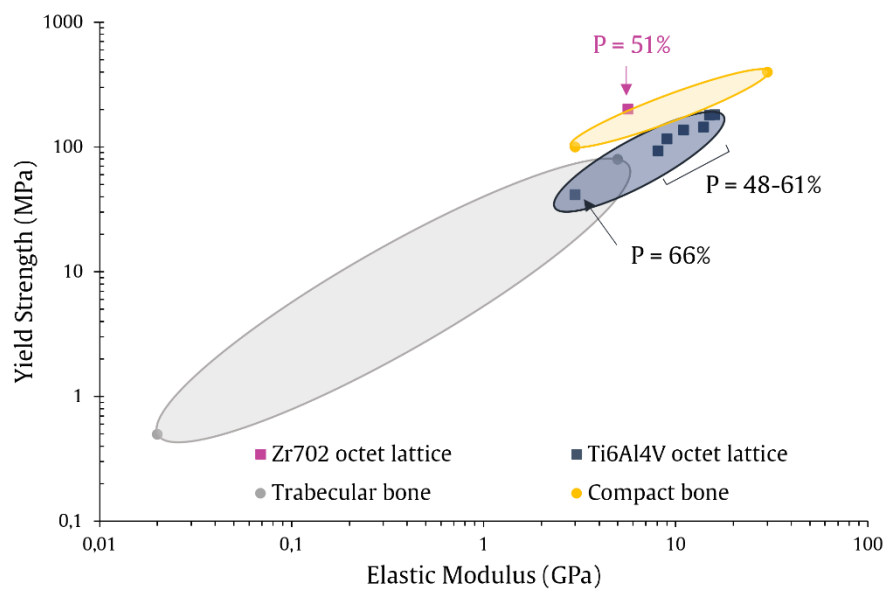


Figure 43 Yield strength versus elastic modulus for the Zr702 octet lattice structure fabricated in this study, Ti6Al4V octet lattices characterised by various porosities (P) investigated by Melançon et al. [10], compact and trabecular bones. In the case of lattice structures, the elastic moduli and yield strengths refer to the elastic gradients and compressive proof strengths, respectively.

Further studies investigating the elastic gradient and compressive proof strength of Zr702 octet lattices with porosities higher than 51% are recommended. As higher porosities are challenging to be obtained with the octet unit cell size chosen in this study (1.1 mm), the author recommends increasing the porosity by increasing the unit cell size of the lattice structure whilst maintaining the same designed strut size.

5.3 Conclusions and recommendations

In this study, the compressive behaviour of both bulk and lattice L-PBF Zr702 was investigated, in order to establish the mechanical properties of the material and the lattice structure, and to assess the suitability of the fabricated lattice structure for orthopaedic implants.

The lattice structure fabricated with the conditions C2 was mechanically tested following the ISO 13314 [93] with the objective to assess its suitability for an orthopaedic application.

The unloading test performed according to ISO 13314 [93] allowed to characterise the elastic gradient of the Zr702 octet lattice printed with the optimal parameters found in this study, and to demonstrate the suitability of the structure for load bearing orthopaedic implants. Indeed, the structure exhibits an elastic gradient of 5.64 ± 0.03 GPa, which is close to the lower limit of the compact bone's stiffness (5-40 GPa).

The mechanical tests also revealed that the structures fabricated by L-PBF with the C2 settings show consistent mechanical behaviour both in the elastic region and in the plastic region (unloading test and preliminary test), suggesting that a good control of the fabrication of the lattice structure was achieved in the manufacturing.

Lower elastic gradients could be achieved by increasing the lattice's unit cell size, which would result in a higher lattice porosity, whilst keeping the same manufacturing process settings.

With respect to the material, it was found that the compressive elastic modulus of L-PBF Zr702 was 94.75 GPa, that is lower than the tensile Young's modulus of the wrought material (~99 GPa). Nevertheless, the compressive elastic modulus is higher than the *tensile* elastic modulus of the L-PBF Zr702 material [7].

The material testing in compression posed several challenges. Among the others, the restriction on using extensometer - which allow for the accurate estimation of the deformations in the elastic regime - when testing materials under compression at the

AFRC. Such challenge was overcome by: 1) coupling the load data of the compression test machine with the DIC analysis, which enabled to track the displacements of single points in the speckle pattern, and thus to calculate the true strain in the material during its compression; 2) introducing cyclic loadings in the test, which allowed to recover the elastic deformations in the unloading steps and to determine the linear elastic regime. Data denoising of DIC-derived true strains was essential to couple accurate deformations with stresses, and to obtain the elastic modulus of the material. As a further improvement of the material testing in compression, the author would recommend performing tests on taller samples, as this would allow to monitor a larger speckle (hence the displacements of more points) during the DIC analysis. Indeed, increasing the number of speckle points (hence displacements at each frame) would result in reduced noise in the output data (true strains).

Chapter 6

Biological performance of Zr702 lattice and bulk material: Results and Discussion

In this chapter, the experimental results of the biological tests performed on Zr702 bulk disks and lattice structures are illustrated and discussed. The cell viability – that is the number of living cells – was assessed on the different substrates (i.e., Zr702 bulk disks, Zr702 lattice structures, and tissue-culture plastic) by MTT assay and by fluorescence imaging.

The chapter is structured in the following sections: §6.1 presents the calibration curve of the MTT assay that enabled the calculation of the viable cells by the measured absorbance; §6.2 presents the results of the MTT assay on the various cellular supports; §6.3 illustrates the results of the fluorescence imaging performed on the various scaffolds; §6.4 discusses the results relative to the MTT assay and the fluorescence imaging; the influence of the scaffold type and the culture time on the cell viability is discussed in the subsections §6.4.1 and §6.4.2, respectively; §6.5 discusses the role of the surface topography of the Zr702 bulk and lattice structure on the cellular behaviour; §6.6 concludes the chapter with the key findings and recommendations for future work.

6.1 Calibration Curve for the MTT assay: linking the measured absorbance to the number of living cells

A calibration curve for the MTT assay was developed to allow the estimation of the living cells through the absorbance of the MTT solution. A series of cell densities was prepared to have a known number of cells (from 6,000 to 60,000) and the MTT assay was performed to measure the absorbance of the relative MTT solution.

The results (reported as average absorbance and their standard deviations) are found in Figure 44. The absorbances reported in the scatter graph are the measured absorbances to which the blank (DMSO) absorbance has been subtracted. As illustrated in Figure 44, the calibration curve enables to estimate the number of living cells (from 6×10^3 to 6×10^4 cells) for values of absorbances that vary between 0.13 to 2.53.

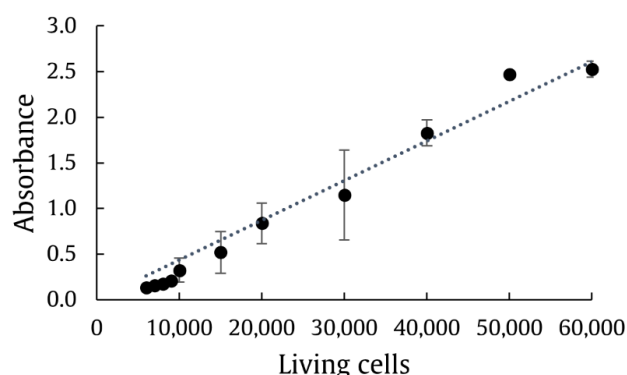


Figure 44 Calibration curve for the MTT assay that links the measured absorbance to the known number of seeded cells and enables the number of living cells to be estimated from the absorbance of the MTT solution.

The linear regression model that was elaborated in Office Excel was based on the average absorbances, and the intercept was set to zero (see the blue dotted line in Figure 44), as the absorbance of the blank was already taken into account. This is equivalent to associate the absorbance of the blank with no living cells.

The linear model that links the absorbance of the MTT assay solution to the number of living cells is shown in Equation 16. The R^2 value for the model was 0.99, indicating a very good correlation with the experimental data.

Equation 16

$$\text{Living cells} = \frac{\text{Absorbance}}{4.35059} \times 10^5 = 22660.59 \times \text{Absorbance}$$

As shown in the graph (Figure 44), most of the cell densities used for the calibration curve gave a consistent and repeatable absorbance (notice the tight standard deviations), which makes the “absorbance-living cells” model reliable. Nevertheless, it can be noticed that for the 30,000 seeded cells group, the absorbance varied significantly (notice its deviation in the graphs), indicating that cells had different metabolic activity in different

experiments. The variability of cell behaviour is a well-known challenge of biological studies in general. Here, these results do not pose particular challenges as the average response is well aligned to the expected gradient of absorbance of the MTT test. Nevertheless, they highlight the challenges of such experiments when it comes to repeatability assessment.

Equation 16 was used to estimate the number of viable cells in the MTT assay performed on the C2 lattices, zirconium disks and plastic control, which are presented in the next section §6.2.

6.2 Results of the MTT assay

The MTT assay was performed on Zr702 lattices (fabricated with the C2 manufacturing conditions), Zr702 bulk cylinders and on tissue-culture plastic, the latter of which was used as a control for the experiments.

In the preliminary tests carried out to validate the method used in this study, it was noticed that the Formazan-DMSO solution changes colour (from purple to blue, as schematically illustrated in Figure 20 in Chapter 3) when it remains in contact with Zr702 for longer than one minute, and that its colour becomes more intense with time. This fact constitutes a significant challenge in the execution of the MTT assay, as the technique is colorimetric and calls for stable-in-colour solutions. To overcome the issue, the DMSO was added and mixed quickly to the wells containing Zr702 samples, and 200 μ L of the solution were withdrawn immediately after and put into a new 96 multi-well plate for the analysis. Once removed from the Zr702, the Formazan-DMSO solution is stable in colour, and can be analysed to estimate the cell viability.

The full results of the MTT essay at 24 hours and 72 hours after cell seeding on different substrates (lattice, bulk, plastic) are reported in Table 20. The absorbances listed there are the measured absorbance to which the blank absorbance (DMSO) has been subtracted.

Table 20 Full results of MTT assay: absorbance at 540 nm. EXP X refers to the experiment X. The values highlighted in red indicate that they are below the lowest absorbance limit of the calibration curve ($abs = 0.13$).

	Lattice		Bulk		Plastic	
	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr
EXP 1	0.666	0.062			0.929	2.247
EXP 1	0.207	0.059			0.963	2.237
EXP 2	0.248	0.101	0.562	0.345	1.189	1.882
EXP 2	0.286	0.222	0.555	0.265	1.179	1.969
EXP 2					1.108	
EXP 3	0.272	0.167	0.499	0.917	0.723	1.664
EXP 3	0.361 *	0.150	0.180 **	0.602	0.789	1.982

* Value discarded from further analyses as the sample reacted instantaneously with DMSO, invalidating the test. Indeed, the suspension colour was dark blue rather than light purple.

** Value discarded from further analyses as this result is underestimated: because some Formazan crystals were poorly attached to the bulk surface, they were withdrawn with the media and did not contribute to the colouring of the DMSO solution.

The average values and relative standard deviations of the absorbance for the different groups were calculated on the valid test results (refer to the comments in Table 20).

Table 21 Summary results of the absorbance measured at 540 nm in the MTT assay and the calculated cell viability. Data are presented as Average $\pm$ Standard Deviation for each group.

Material	Absorbance (-)		Cell viability (-)	
	24 hr	72 hr	24 hr	72 hr
Lattice	0.336 $\pm$ 0.167	0.127 $\pm$ 0.059	7,613 $\pm$ 3,784	2,878 $\pm$ 1,336
Bulk	0.539 $\pm$ 0.028	0.532 $\pm$ 0.255	12,214 $\pm$ 634	12,055 $\pm$ 5778
Plastic	1.021 $\pm$ 0.154	1.997 $\pm$ 0.202	23,136 $\pm$ 3,490	45,253 $\pm$ 4,577

The number of viable cells on each material at 24h and 72h were calculated with Equation 16. The results are reported in Table 21 and are graphically shown in Figure 45. At 24 h from the cell seeding, 2.3×10^4 cells were found (on average) on the tissue-culture plastic supports, whilst 7.6×10^3 cells and 1.2×10^4 were calculated for the zirconium lattice and the zirconium bulk cylinders, respectively. At 72 h of cell culture, the average number of cells on plastic was calculated at 4.5×10^4 cells. At the same time interval, the average cell viability on the zirconium lattice and bulk were 2.9×10^3 cells and 1.2×10^4 cells, respectively.

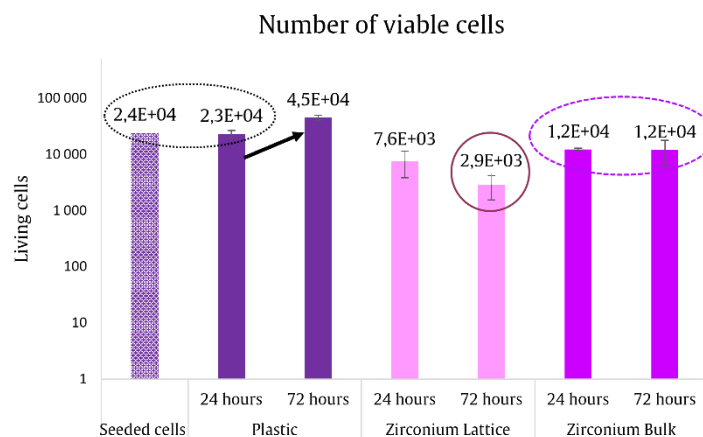


Figure 45 Estimated viable cells on the different substrates (Plastic, Zirconium Lattice and Bulk) compared to the target number of seeded cells (the number refers to the cells seeded per well or per substrate). Notice this is equal to the number of viable cells on plastic at 24h from seeding, which indicates that the experiments were well executed. Red circle highlights values lower than 6,000 cells (the lower limit of the calibration curve). Fuchsia circle highlights the substantial invariability of the number of viable cells on bulk zirconium at 24h and 72h.

As the detailed discussion of these results is left for the dedicated section (§6.4), the author would like to highlight here that the number of viable cells on plastic after 24 h from seeding (23,000 on average) is well in line with the target value of 24,000 seeding cells (see Figure 45). This indicates that the experiments were well executed: cells were well-counted and well-dispersed in the media before being seeded onto the different substrates. This result gives confidence that the MTT readings on the other substrates are representative of the “reality”: the eventual differences in the measured absorbance would be due to the effects of the substrate, whilst uneven seedings can be excluded.

6.3 Results of the fluorescence imaging

Fluorescence imaging was used with a kit Live/Dead to stain with different fluorescent markers the living and the dead cells: Calcein Green stains the living cells, whilst Propidium Iodide stains the dead ones, which appear as fluorescent red. Representative microscopies for the different cell substrates (zirconium bulk, zirconium lattice, and tissue-culture plastic) taken after 24 hours (within light green boxes) and 72 hours (within dark green boxes) from the seeding can be found in Figure 46.

Cells seeded onto *tissue-culture Plastic* (Figure 46.14-15) adhered evenly on the surface, and were all living after 24 hours of culture, as only green cells were found on this substrate. The morphology of these cells was generally elongated and stretched, whilst some (green) round cells were spotted on top of stretched cells. The microscopies taken at 72 hours (Figure 46.15) were characterised by a significantly higher cell density compared with those relative to 24 hours, with the plastic surface being fully covered with cells (condition of “full confluence”). At both times of observation, cells gave intense green fluorescence.

Cells seeded onto *Zirconium Bulk* (Figure 46.1-5) adhered to both the top of the metallic material (see “A” area, Figure 46.1) and the plastic Petri dish (see “B” area, Figure 46.4) after 24 hours from seeding. Cells that adhered to zirconium were unevenly distributed on the sample, with areas covered by adhered cells (such as areas close to the centre of the sample, like in the “A” area - Figure 46.1-2) and others – typically close to the sample’s edges – where cells were hardly or not at all found. Cells that had adhered on the top of the Zirconium Bulk were alive at both 24 hours and 72 hours (see the “A” areas in Figure 46.1-2, where mostly fluorescent green cells are found). The cell morphology was generally stretched. Interestingly, cells exhibited a preferential orientation (as highlighted by the white arrows in the microscopies in Figure 46.1-2 and Figure 47): cells were aligned with the grooves that were left on the surface by the grinding operations that were performed during the sample preparation. These grooves can be better appreciated in Figure 47, where they can be spotted by the fluorescence reflections in the merged channels microscopies (Figure 47.a) or in the split channels microscopies with increased contrast and brightness (Figure 47.b-c).

The number of viable cells on the top of the bulk samples did not vary significantly by passing from 24 hours to 72 hours from seeding. These results are in agreement with the MTT assay. Cells that adhered to the plastic support (Figure 46.4-5) were viable, with the vast majority of them being green fluorescent at both 24 hours and 72 hours and

exhibiting a stretched morphology. Interestingly, the cell morphology tended to be more circular when cells adhered to areas close to the metallic samples (Figure 46.3). The number of cells on plastic increased when passing from 24 hours to 72 hours after the seeding (see Figure 46.4-5).

In the case of the *Zirconium Lattice* (Figure 46.6-13), cells were mostly found attached to the plastic bottom of the Petri dish (Figure 46.9 and Figure 46.11), whilst few cells adhered onto the top, the internal features (Figure 46.6-7) and bottom sides (Figure 46.11-12) of the lattice. The viability of the cells varied depending on the particular surface (hence material) that cells came in contact with. Cells that adhere to the top flat ground surfaces were intense fluorescent green (Figure 46.6), hence viable and well attached to the material. Interestingly, cell morphology was not stretched (as it happened for bulk zirconium), but rather circular in shape, albeit spread onto the surface. Furthermore, no preferential orientation was noticed. This cell morphology was rather similar to the one observed by Aristizabal *et al.* [7] on bulk zirconium after three days of culture, as it can be seen in Figure 48. Cells that adhered to the internal features of the lattice (i.e. internal struts and nodes) exhibited mild red fluorescence at 24 h, and intense red fluorescence at 72 h from seeding (Figure 46.7) and were characterised by a circular cell morphology.

Cells that adhere to the outer regions of the Petri dish were fluorescent green and exhibited the classic morphology of plastic-adhered cells at both time frames (Figure 46.9-10). Furthermore, the number of viable cells increased with time, as it can be noticed by comparing Figure 46.9-10. At both time frames, cells that adhered to the tissue-culture Plastic by falling from the upper portion of the lattice and that were trapped between the plastic and the metallic sample, as those in “D” areas (Figure 46.13) or in the “C” areas (Figure 46.8, Figure 46.11-12), gave intense red fluorescence and exhibited a circular morphology, indicating cellular death.

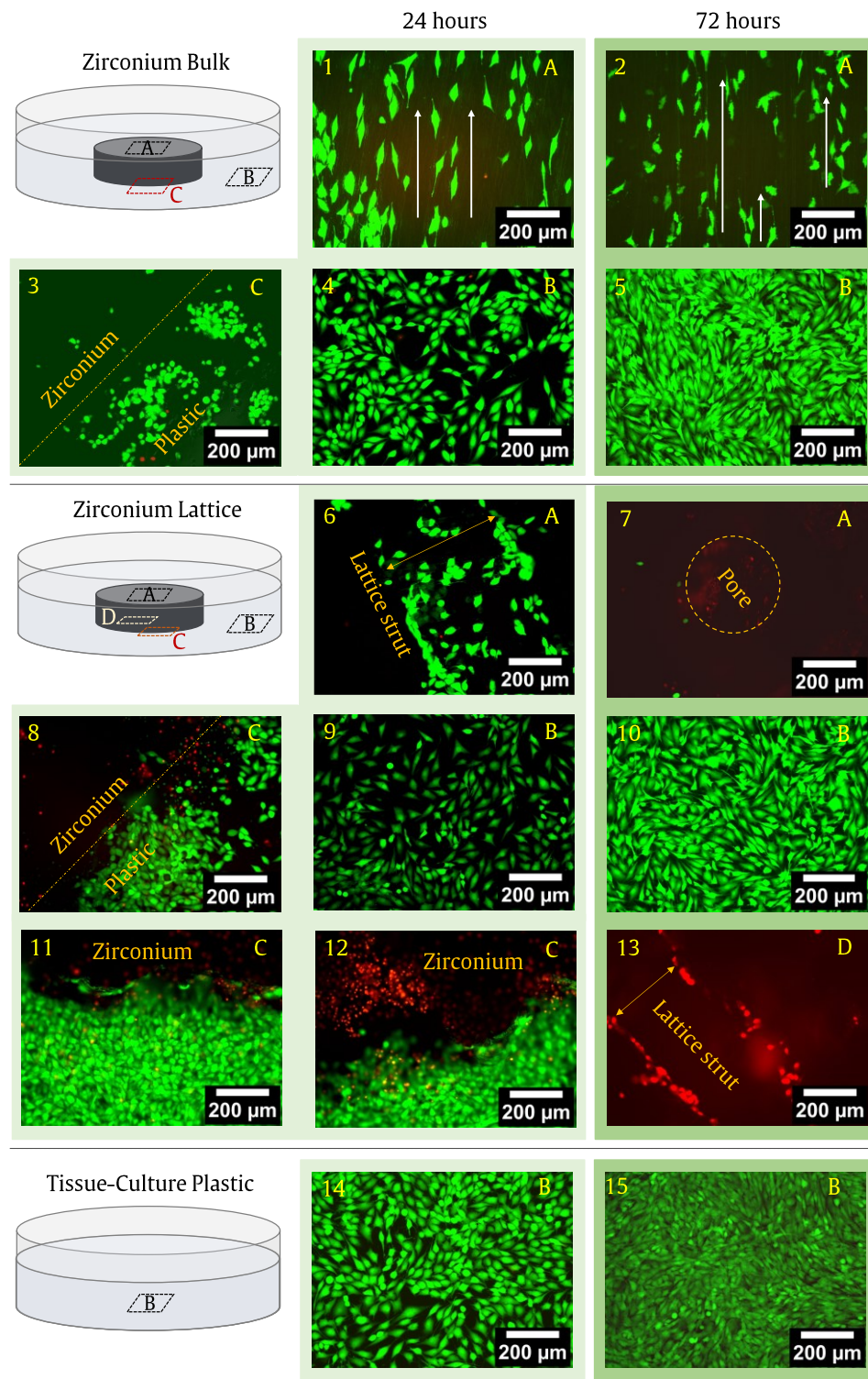


Figure 46 Microscopies of cells stained with fluorescent markers. Results are reported by substrate type (Zirconium Bulk, Zirconium Lattice and tissue-culture Plastic) and time frame (24 hours, in light green boxes; 72 hours, in darker shade green boxes). Different letters indicate different areas of the sample.

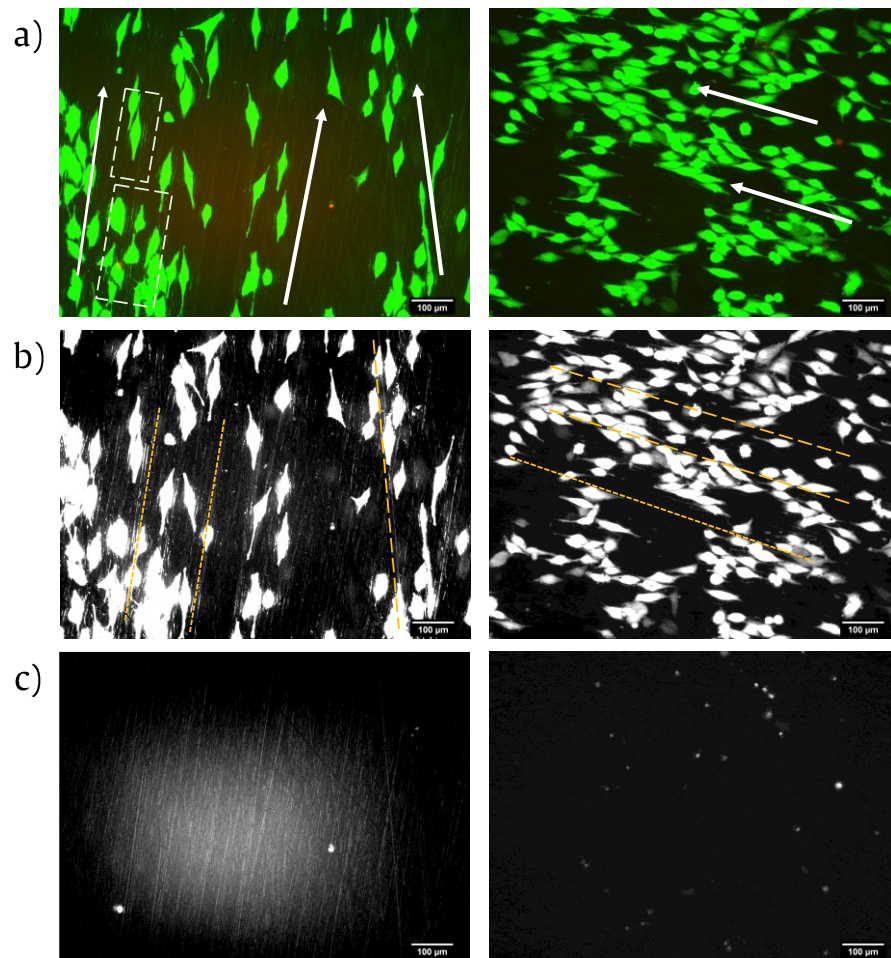


Figure 47 Effect of the surface topography on cell orientation on bulk samples: alignment of living cells with surface grooves. White arrows indicate the cell orientation whilst boxes and lines highlight the surface grooves (a, b). Green (b) and Red (c) channels - with increased brightness and contrast - allow to better appreciate the surface topography and the cell orientation.

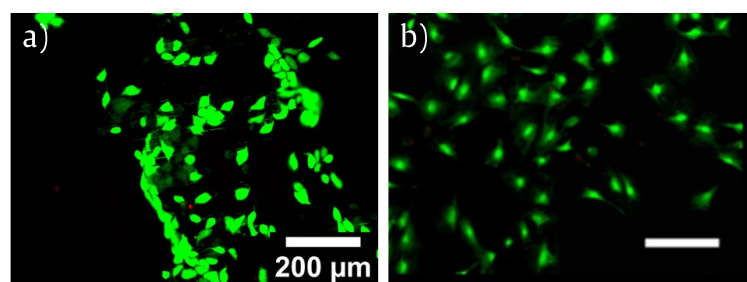


Figure 48 Comparison on the cell morphology of cells seeded onto zirconium lattice investigated in this study (a) and those onto bulk zirconium (b) reported by Aristizabal et al. [7]. Cells had adhered and spread with similar morphology and with no preferential orientation. Scale bar is 200 µm for both the microscopies.

6.4 Discussion of the MTT assay and fluorescence imaging results

In this section, the results relative to the cell viability that was evaluated by MTT assay and by fluorescence imaging are analysed and discussed. In particular, the results of the MTT

assay are analysed statistically to assess the effects of the substrate (zirconium bulk, zirconium lattice, and plastic) as well as the time of cell culture (24 h and 72 h) on the cell viability. The results obtained from the two tests (MTT assay and fluorescence imaging) are compared.

6.4.1 The effect of the substrate on the cell viability

The potential effect of the substrate (zirconium bulk, zirconium lattice, and plastic) on the cell viability was investigated with the statistical analysis of the MTT assay results and with the microscopies obtained from the fluorescence imaging.

With respect to the statistical analysis of the measured absorbance from the MTT assay, it should be noted that the Levene's test was not performed given the small sample size for each group. Therefore, the condition of homoscedasticity was excluded. The Welch's ANOVA and Games-Howell's post-Hoc test (p -value = 0.05) was performed to assess whether the difference between the means of the absorbance at specific times of cell culture (24 hours and 72 hours) between substrate groups (Plastic, Bulk, Lattice) was significant.

Table 22 Welch's ANOVA on the Absorbance versus Substrate at 24 hours and 72 hours cell culture duration. N is the sample size of each group; Mean is the average absorbance measured for each group.

Time	p-value	Substrate	N	Mean
24 hours	0.001	Bulk	3	0.539
		Lattice	5	0.336
		Plastic	6	1.201
72 hours	0.000	Bulk	4	0.532
		Lattice	6	0.127
		Plastic	6	1.997

As shown in Table 22, a statistical difference in the means between the different substrates was found with Welch's ANOVA both at 24 hours and 72 hours in the cell culture duration,

with p-values of 0.001 and 0.000 for 24 hours and 72 hours, respectively. This result meant that there was at least a statistical difference between two groups. The Welch's ANOVA cannot discriminate which pairs are statistically different from those which are not. Therefore, the Games-Howell's post-Hoc test was performed in order to find the statistically different pairs. As shown in Table 23, the couples that shown statistically significant difference were Plastic vs Bulk and Plastic vs Lattice, at 24 hours and 72 hours (p-values are reported in Table 23). However, the pair Lattice-Bulk did not show statistically significant difference when cells were cultured for 24 hours or 72 hours on these substrates (p-values > 0.05, see Table 23). This means that MG-63 cells cultured on bulk or porous Zirconium do not show significantly different metabolism at least until 72 hours have passed. Based on the statistical analysis of MTT experiments, these cells seem not to prefer one form of Zirconium over the other.

Table 23 Pairwise comparisons with the Games-Howell's post-Hoc test of the Absorbance versus Substrate. At fixed time levels (24 hours and 72 hours), the p-value relative to the difference between each pair of levels in Substrate is given. The differences are statistically significant for p-values ≤ 0.05 .

Time	Difference of levels	Adjusted p-value	Statistically significant
24 hours	Lattice - Bulk	0.147	
	Plastic - Bulk	0.002	✓
	Plastic - Lattice	0.001	✓
72 hours	Lattice - Bulk	0.134	
	Plastic - Bulk	0.001	✓
	Plastic - Lattice	0.000	✓

The results are visualised in the "Boxplot and whiskers" charts for the absorbance vs Substrate at 24 hours (Figure 49.a) and at 72 hours (Figure 49.b). As it can be seen from Figure 49, cells cultured on plastic showed a significantly higher absorbance compared to then one of zirconium bulk and lattice, at both 24 hours and 72 hours of cell culture.

Therefore, the cells cultivated on plastic exhibit higher metabolism compared to those cultivated on zirconium (lattice or bulk).

These results are well in line with the observations made at the fluorescence microscope. By comparing the cell density of green (hence viable) cells on plastic (Figure 46.14), zirconium bulk (Figure 46.1), and Figure 46.6) at 24 h, it is clear that plastic is able to support the cell adhesion of a higher number of cells. However, when comparing the cell viability on zirconium bulk and lattice, the differences do not seem significant, at least at 24 h (compare for example Figure 46.1 with Figure 46.9, or Figure 46.3 with Figure 46.8). The graphs in Figure 49 also show the different variability of the absorbance in bulk and lattice groups, at 24 hours and 72 hours, albeit in an opposite way: at 24 hours, the results relative to the bulk were less dispersed than the lattice, whilst at 72 hours the opposite situation occurred. As demonstrated before (Table 23), the results of the MTT assay relative to the bulk zirconium and the lattice zirconium did not exhibit statistically significant difference, as the null hypothesis (H_0 : the mean absorbance of the groups are equal) cannot be rejected due to p-values greater than 0.05 (Table 23).

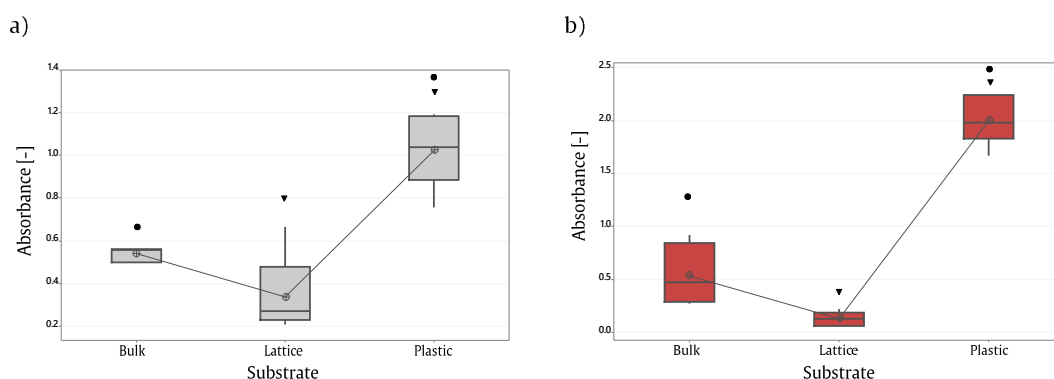


Figure 49 Boxplot and whiskers charts for the Absorbance versus Substrate measured at 24 hours (4a, grey boxplot), and at 72 hours (4b, red boxplot) to highlight the eventual effect of the substrate. Groups that share the same marker exhibit statistical difference under the Welch's ANOVA - Games-Howell's post-Hoc test (p -value = 0.05).

One might question the reliability of the Games-Howell's post-Hoc test results for the difference of levels "Lattice-Bulk", given the different data dispersion. Since the Games-Howell's post-Hoc test is a non-parametric statistic test, the different data dispersions

should not pose a concern on the reliability of the test. Furthermore, this test is more prone to Type I errors (rather than Type II errors), meaning that it is more prone to reject the null hypothesis when it is true, rather than accepting it when it is false. As the “Lattice-Bulk” difference resulted non-significant, the Type I error is excluded.

Nevertheless, the limited sample size of the study (particularly for the Bulk group, see Figure 49) poses a significant challenge in the statistical analysis of the obtained experimental data. Eventual outliers in the data cannot be identified, and this may have impeded to appreciate an eventual statistical difference between the bulk and lattice groups.

This consideration is particularly relevant to the test results relative to the 72 hours (refer to Figure 49.b), as the mean absorbance for the bulk and the lattice are fairly different (0.532 for the bulk and 0.127 for the lattice, as shown in Table 22), but the intervals of confidence overlap (see Figure 49), which led to the rejection of the test hypothesis. The observations at the fluorescence microscope (the bulk in Figure 46.2, and the lattice in Figure 46.7) would seem to suggest that there is actually a difference in the cell behaviour when MG-63 cells are seeded on zirconium bulk or zirconium lattices. Indeed, MG-63 cells on bulk zirconium exhibited intense green fluorescence at 72 h (Figure 46.2) and stretched morphology, indicating a good state. On the other hand, MG-63 cells on the lattice exhibited lower intensity in green fluorescence or red fluorescence, indicating either cell sufferance or death.

It is interesting to notice, however, that although cells seemed to be less viable on zirconium lattice compared to the bulk (at 72h), the cells that were cultivated in the same Petri dish but adhered to the plastic at 24 h actually exhibited intense fluorescent green, indicating that they were in good health. Furthermore, by observing the positive trend in cell density on the “B” locations¹² for the bulk and lattice samples (Figure 46.4-5 and

¹² The reader might wonder why, if MG-63 cells are capable of replicating on the plastic surfaces of the Petri dish that contains zirconium samples, the MTT assay gives such low absorbance values compared to plastic. The

Figure 46.9-10, respectively), it can be concluded that the zirconium material (in either bulk or lattice form) does not leach cytotoxic substances. As such, the author concludes that the zirconium lattices do not have cytotoxic effects *per se*, and that the eventual difference in cell viability with zirconium bulk (therefore, material) at different times of cell culture is to be researched elsewhere.

Having excluded potential cytotoxic effects of leached substances from the zirconium lattices, the author proceeded in exploring different hypotheses that would explain the reasons behind the difference in the number of living cells onto two substrates that have the same chemical composition.

The first hypothesis is related to the lattice's architectural porosity. As this was 51%, one may suggest that - at 24 hours - the number of adhered cells is half compared with the bulk material because there is only half of the top surface available for the seeded cells. Nevertheless, it is to be noted that the cell suspensions were able to sit on the top surface only for seconds, before falling within the lattice pores. As cells need hours to adhere to a surface, it is highly unlikely that cells were actually able to adhere on the top surface, occupying the equivalent of half of the full disk top surface.

Instead, the author believes that the cells falling into the lattice follow a different destiny, depending on the material/surface to which they come into contact and attach to. Indeed, although cells are deposited on the top lattice surface, the cell suspension "drop" breaks after seconds due to the lattice pores, making the cells penetrate through the porous metallic structure and deposit either on the plastic or the inner metallic structures.

Cells that fall into the lattice and adhere to the internal lattice features would interact with more complex topographies, which might hinder their capacity of adhering and proliferating. This hypothesis is corroborated by the lower intensities in green fluorescence and increased red fluorescence (especially at 72 h). Although not ideal from

reason for this is that the MTT assay was performed on 24-well plates, in which the zirconium samples covered the ~64% of the well area and therefore, the contribution of cells that adhere to the plastic portion results negligible.

a manufacturer's point of view, this issue may be overcome by chemically treating the zirconium lattices and porous features in general, in order to smooth the surfaces, by removing partially melted particles, ridges and laser weld tracks.

Cells which fall to the bottom of the lattice sample, adhere to the bottom of the Petri dish. These are therefore covered by the metallic material on top and would not get access to fresh and adequate amounts of media: it is likely that nutrients would be hardly delivered in these areas, and cellular by-products cannot be taken away. The author believes that cells in these areas are killed by their unfortunate location, as they are trapped between two materials, and not by the metallic material (or lattice) itself.

6.4.2 The effect of the time of culture on the cell viability

The potential effect of the duration of the cell culture (24 h and 72 h) on the cell viability was investigated with the statistical analysis of the MTT assay results and with the microscopies obtained from the fluorescence imaging.

The "Boxplot and whiskers" chart in Figure 50 shows the absorbance for each group, clustered by substrate and coloured according to the lasting of the cell culture (24 and 72 hours). The groups exhibited different variability in the measured absorbance, as demonstrated by the different size of the boxplots (notice, for example, the difference between the boxes relative to the Bulk at 24 hours and 72 hours).

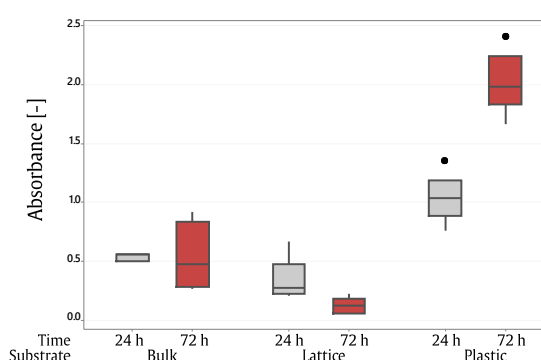


Figure 50 Boxplot and whiskers graph of the absorbance for the different substrates (zirconium bulk, zirconium lattice, and tissue-culture plastic) and cell culture duration (Time - 24 hours and 72 hours). The boxplots of the groups at 24 hours and 72 hours are coloured in grey and red, respectively. Groups that share the same marker are statistically different according to the Welch's ANOVA and Games-Howell's post-Hoc test (p -value = 0.05) for the same substrate.

The Welch's ANOVA and Games-Howell's post-Hoc test (p-value = 0.05) was performed to assess if the mean absorbance of the MTT solution was affected by the duration of the cell culture (24 or 72 hours), on the same type of substrate. The statistical test revealed that the absorbance at 24 and 72 hours were statistically different only on the cell-tissue culture plastic substrate (see Figure 50), with an increase in the absorbance when passing from 24 to 72 hours in the cell culture duration (p-value = 0.000).

The variations in the mean value for Zirconium (both in the shape of full disk and lattice) were not significant, with p-values being 0.968 and 0.065 for bulk and lattice, respectively.

The detailed results or the statistical tests are reported in Table 24.

Table 24 Results of the statistical analysis of the absorbance versus time (cell culture duration), for each substrate. N is the sample size of each group, Mean is the average absorbance measured for each group, and p-value and Adjusted p-value are the statistical test response of the Welch's ANOVA and Games-Howell's post-Hoc test, respectively.

Substrate	Welch's ANOVA	Games-Howell's post-Hoc test			
	p-value	Time	N	Mean	Adjusted p-value
Plastic	0.000	24 hours	6	1.021	0.000
		72 hours	6	1.997	
Bulk	0.968	24 hours	3	0.539	0.968
		72 hours	4	0.532	
Lattice	0.065	24 hours	5	0.336	0.065
		72 hours	6	0.127	

The positive trend of the absorbance with time that was observed on plastic indicates an increase that the adhered cells had replicated on the plastic, contributing to an increase in the number of highly metabolically active cells on the surface. This is an indication of the good state of the adhered MG-63 cells. The same conclusions were drawn from the microscopies obtained with the fluorescence imaging. At 24 h (Figure 46.14), the cells exhibited intense green fluorescence and stretched morphology, indicating a firm cell adhesion. The rounded cells that were spotted on top of the stretched cells are replicating

cells. The higher cell density found at 72 h (Figure 46.15) corroborates the observed increase in the measured absorbance from the MTT assay and confirms that cells had replicated on this support from 24 h to 72 h. This outcome was expected, as the tissue-culture plastic is a material that is biocompatible by design and was selected as control group for this *in vitro* study.

In the case of zirconium bulk, instead, the results suggest that increasing the time of cell culture from 24 hours to 72 hours does not lead to substantial variation in the metabolic activity of cultured cells onto this type of substrates. In fact, approximately the same average number of cells was found. This might indicate that the metallic material supports cell adhesion but does not support cell proliferation of MG-63 cells in this time period. Hence, although cells did replicate on plastic, the number of viable cells in Petri dishes with Zirconium Bulk is lower compared with the control (tissue-culture plastic), at 72 hours. This behaviour may be due to a number of reasons and may be related to the particular interaction between the metallic material and the cell line. For example, the cell type used in this study (MG-63) may need more time to become metabolically active or to replicate on zirconium. Further investigations that would extend the cell culture duration to five and seven days and increase the sample size (between ten and twenty samples for each group) would be recommended to shed light on the behaviour of cells when in contact with Zirconium, both in bulk and porous forms.

With respect to the lattice, the average cell viability decreased when cells were cultured for 72 h compared to 24 h. Although the difference resulted statistically non-significant in the MTT test, the observations made at the fluorescence microscope (Figure 46.6 and Figure 46.7) suggest that the decrease in the cell viability is substantial.

The author would like to emphasise once again that the time frame of this study is limited to an observation period of only 72 hours, a period of time in which cell adhesion is dominant over other cellular activities such as proliferation and migration [183]. As shown by Torres-Sanchez *et al.* [183], who investigated the effect of porosity and pore size on cell

viability over a longer period of time (from 1 to 12 days), metallic scaffolds with pores in the range of 300-500 μm exhibit lower cell adhesion compared with bulk disks at 24 and 72 hours of culture, albeit with non-significant differences, as it was observed in this study. However, after 12 days of culture, these porous structures exhibited significantly higher cell viability than the bulk disks. Therefore, the author concludes that, whilst the zirconium lattice tested here does not improve the early biological performance of the material in 72 hours, we should not exclude that the structure might outperform at longer observation periods.

In addition to this, the author would like to reflect on the difference in the cell behaviour when comparing the evolution of the cell viability (in time) for zirconium bulk and lattice structures. Following the considerations made in section §6.4.1 on the cell viability on the zirconium bulk and lattice structures, the author believes that the surface topography of the internal lattice features plays a role on the cell viability and encourages others to further investigate this hypothesis.

6.5 The role of the surface topography of Zirconium bulk and lattices on the cell viability

In this section, the author expands on her hypothesis on the potential role of the surface topography of zirconium bulk and lattices on the cell viability at 24 h and 72 h of cell culture.

Such hypothesis was formulated by the gathered fluorescence microscopies, which enabled the determination of the location of viable and dead cells on the substrates, as well as to recognise several topographical features on the different regions of the lattice and bulk samples. For example, it was noticed that the top surface lattice was flat and smooth in appearance, whilst the internal surfaces (visible through the upper pores) were curved, exhibited wavy texture (weld-tracks coming from the fusion of subsequent zirconium layer in the manufacturing) and micrometric spherical protuberances (partially melted particles) that made the topography particularly complex and irregular. On the

other hand, the bulk surface topography was characterized by straight grooves that originated in the grinding operations during the sample preparation.

The author suggests that the bulk's topographical features guided the cell adhesion, as the cells were stretched and aligned with the grooves, as it was previously shown in Figure 47. This phenomenon is explained by the mechanism of "contact guidance", which is well described in the excellent review of Anselme *et al.* [125].

With respect to the lattice, it appears that the flat ground top surfaces do not guide the cell adhesion and spreading in the case of the lattice, as illustrated in Figure 48. In this case, indeed, no significant grooves were observed on the sample surface. Therefore, the author suspects that the surface topography did not exhibit the topographical clues that would orient the cells during the cell adhesion.

The internal features such as lattice struts – which are characterised by curved and irregular surfaces – were populated by dead cells (consider Figure 46.7).

Following the conclusions made in §6.4.1, the author suggests that the surface topography of the various substrates played a role in the cell behaviour and ultimately determined the viability of adhered cells. More precisely, she presumes that MG-63 cells that attach onto flat ground surfaces adhere firmly to the material and spread onto it, maintaining the cell viability. On the other hand, MG-63 cells that fall onto curved and highly irregular surfaces – such as lattice struts and nodes – are only able to poorly adhere to these, with a consequent decrease in metabolic activity and, at 72 h of culture, cell death.

This hypothesis is in line with the literature [125, 126, 138], although these studies were made on flat continuous surfaces.

Jamshidi *et al.* [138] reported that human osteosarcoma¹³ cells did not spread onto as-manufactured L-PBF Ti6Al4V plane surfaces but rather kept a circular shape or maintained a "sedimental stage". Interestingly, the metabolic activity of cells attached to these

¹³ Human osteosarcoma cells are the cell type used in this study as well.

surfaces – measured through colorimetric assay (with Alamar Blue)– was lower on these surfaces, compared to etched (hence characterised by a lower average areal surface roughness) surfaces. Jamshidi *et al.* [138] assumed that the limited or absent cellular spreading onto the as-manufactured L-PBF surfaces is due to the presence of partially-melted particles, as these would reduce the space available for the cells to spread.

Anselme *et al.* [125] discussed the research carried out on the effects of surface topography on the adhesion MG-63 cells, concluding that: 1) flat surfaces (in Ti6Al4V) that exhibited grooves were able to orient the MG-63 cell adhesion; 2) “coarse” surfaces were colonised by MG-63 cells at lower levels and with lower “attachment levels” than flat surfaces; 3) the sensitivity to surface topography is significant for MG-63 cells, however it might be lost when testing bone cell lines that exhibit a “more differentiated maturation state than the MG-63 cells” [125].

6.6 Conclusions and recommendations

This study was aimed at assessing the cell viability (i.e., the number of living cells on zirconium and zirconium lattice structures after 24 and 72 hours from the cell seeding. Tissue-culture plastic was used as substrate control for the biological tests. The MTT assay and the Live/Dead Assay (fluorescence imaging) were used to quantify and assess the cell viability on the different substrates.

In summary, the results indicate that, at 24 hours, the cell viability was significantly higher on the tissue-culture plastic than the zirconium substrates. The difference between the bulk and the lattice results were non-significant, although fewer (viable) cells were found on the lattice structure. At 72 hours from cell seeding, the viable cells on the plastic substrate had doubled with respect to the 24 hours reading. Regarding bulk zirconium, the average number of viable cells was comparable with the one found at 24 hours (although a higher deviation was noticed at 72 hours), showing that MG-63 cells can adhere onto flat zirconium disks, and that their viability is stable at least until 72 hours. The cell viability on zirconium lattice after 72 hours fell below the lower limit of the MTT assay's

calibration curve, indicating a significant decrease in the number of living cells. Indeed, a significant fraction of dead cells were observed at the fluorescence microscope.

With respect to the methodology used, the author would like to emphasise the importance of using two complementary assays in biological tests. Not only fluorescence microscopy confirmed the results of the MTT test, but also it allowed to gain better understanding of the cell behaviour on Zr702 in bulk and lattice form. Indeed, by locating the cells seeded onto the different substrates, it was possible to appreciate the cell viability, density and morphology on different sample areas.

By adopting this approach, the author was able to suggest how the surface topography might have played a role in the cellular adhesion on bulk and lattice zirconium. Indeed, it was proposed that grinding-originated grooves on the bulk zirconium's flat surfaces affected the cell adhesion and spreading, by guiding the deposition of the actine filaments. The observations made at the microscope allowed also to propose an explanation to the low absorbance (compared to the control, in the MTT assay) relative to the lattice structure.

Three hypotheses were proposed, depending on the location of the cells in the sample. The first, that is relevant to the cells that attached to the flat top surfaces of the lattice, proposes that MG-63 cells are able to adhere well to these surfaces and maintain their metabolic activity. The second, that is applicable to the internal features of the lattice, assumes that the complex, highly irregular, as-manufactured surface topography of lattice struts and nodes hinder a good cell adhesion, which in turn results in low metabolic activity for MG-63 cells, and leads to cell death. The third is relevant to the cells on the bottom of the lattice that were found dead: in this case, the author presumes that cells were killed by the lack of fresh media availability.

Finally, the author concludes that zirconium is indeed biocompatible and that L-PBF lattice structures need to undergo a chemical (or electrochemical) treatment aimed at smoothening the internal surfaces, to maintain the material's biocompatibility.

The author acknowledges the limitations of this experimental study in relation to the limited sample size and would like to invite others to proceed on the route here marked and use these preliminary results as a basis for further research.

Chapter 7

Surface topography of lattices: Results and Discussion

In this chapter, the results of the analysis of the surface topography of lattices fabricated by L-PBF at different manufacturing settings are analysed and discussed. The primary objectives of this work were to propose and assess an original method for the detection and characterization of partially melted particles (PMPs), which are typical of L-PBF manufactured surfaces and have given rise to concerns over the biocompatibility of L-PBF lattices.

At first, the surface topographies captured by Focus Variation Microscopy are qualitatively described. Then, the results relative to the particle detection and characterization (particle size and distribution) are illustrated. The particle detection method is then discussed in relation to the main methodological choices and the results obtained from the analysis of the lattice surface topographies. The robustness of the method of detection is assessed, highlighting the method's strengths and limitations. The opportunity to characterise lattice surface topography by the particle density parameter is discussed. Finally, the lattices' surface topographies are discussed with respect to the manufacturing process and the functionality of the surfaces in a biomedical perspective. The chapter concludes with the key findings and recommendations for future research in this area.

7.1 Surface topography of lattices measured with Infinite Focus Variation Microscopy

The surface topography of lattices manufactured with different process settings (C1-C6 and L1, L2, L4, L5, L6 and L8) was measured by Focus Variation Microscopy (FVM). The two

groups of manufacturing conditions – from which the lattices are named after – are going to be referred to as CX* and LX* for brevity.

Figure 51a shows an example of the topographical data acquired in this study by FVM, in both 3D and 2D view (top view).

As the reader may notice, the interpretation of such data at first sight can be rather challenging: it is not immediate to recognise the different geometrical features of the octet lattice (upper horizontal struts, nodes, re-entrant diagonal struts) when they are captured by FVM (especially when observing the 2D view). Figure 51 provides the keys of interpretation for these data by linking the topographical data acquired by FVM to the volumetric X-CT data relative to the full lattice structure.

As indicated by the bumpy orange line in Figure 51b, the horizontal struts and the connected nodes actually belong to offset planes, with nodes occupying a higher height profile (Z position) compared to the struts. This indicates that the highest features visible in FVM data (coloured in red in the 3D view, Figure 51a) are actually only the upper nodes. The horizontal nodes, instead, are coloured in yellow according to the height profile (refer to the 3D view and the relative colour map in Figure 51a). Finally, the re-entrant features (45°-oriented struts) connected to the upper nodes are coloured in green, whilst features further below are coloured in blue.

The horizontal cylindrical-like struts, the joints (nodes) and re-entrant features can be also identified in the 2D view of the surface topography (Figure 51a, on the right-end sight), thanks to the specific way each feature reflects the light. From the superimposed topographical data on the lattice structure (reconstructed by X-CT) in Figure 51c, it becomes evident that the black net in the 2D view captures the horizontal struts with the connected nodes, whereas the pinkish areas correspond to the re-entrant lattice struts. These features appear in different colour not only because of their different geometrical orientation, but also because of their peculiar surface topography: whilst the horizontal struts and the connected nodes are characterised by rather “smooth” and thus reflective

surfaces, the re-entrant struts are characterised by uneven and jagged surfaces, which tend to scatter the light. This results in the horizontal struts and the connected nodes appearing as black (due to the light reflection), whilst the re-entrant features appear pinkish.

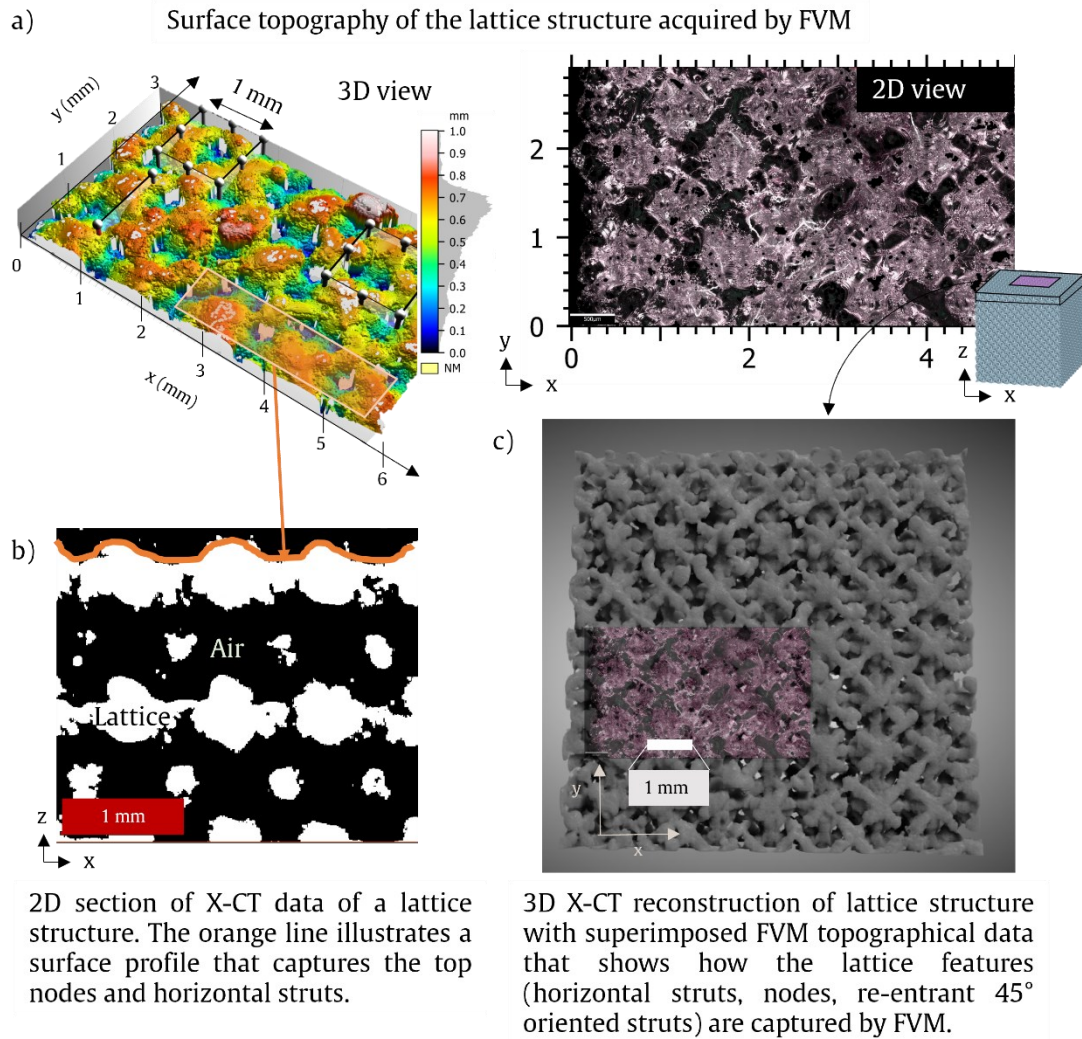


Figure 51 Representative example of topographical data acquired in this study by FVM: a) shows the 3D and 2D views of the topographical data; b) illustrates a single surface profile from an X-CT slice and how it relates to the 3D topographical data acquired; c) helps understanding how the lattice features acquired by FVM are represented and visualised: the black grid of the 2D view corresponds to the upper horizontal struts and nodes, whilst the pinkish areas correspond to the re-entrant features (45° oriented struts).

The topographical dataset shown in Figure 51 is relevant to the lattices fabricated by CX* settings, and describes the maximum lattice surface that can be measured by FVM, also here referred to as “fully exposed outer lattice surface”. This is composed of the upper

exposed surface (i.e., the portion of surface that included only the horizontal struts and nodes, and that appears black in the 2D view), and the *lower exposed surface* (i.e., the 45-degrees re-entrant lattice struts that are connected to the upper lattice nodes, and that appear pinkish in the 2D view). The definitions just introduced are clarified in Figure 52a. The topographical datasets of the LX* group, instead, represent the sole upper surface topography. In this batch of analysed lattices, indeed, the topography was acquired over a depth that allowed to capture only the horizontal struts and nodes.

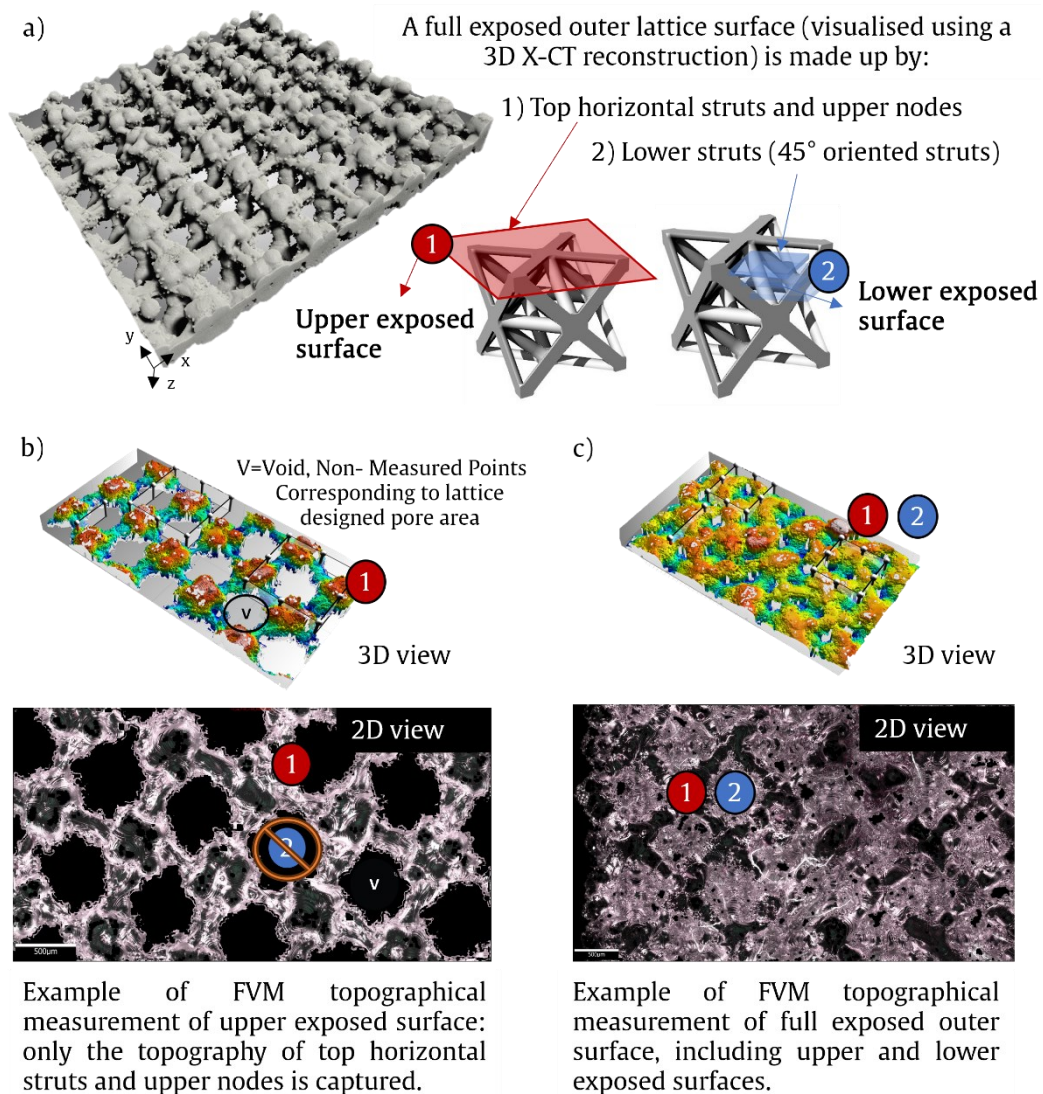


Figure 52 a) Illustration of the “full exposed outer lattice surface” and its components: the upper exposed (outer) surface and the lower exposed (outer) surface; b) and c) show the different lattice features that can be measured by FVM by acquiring the topography of the upper exposed surface only (b) and of the full exposed outer surface (c). The acquisition methods illustrated in b) and c) were used in the measurements of the lattices LX* and CX*, respectively.

A representative example of these datasets is shown in Figure 52b. This measurement method allowed to accelerate the acquisition of the surface topography and to capture PMPs on the side surfaces of the upper lattice struts and nodes.

The particle detection algorithm that was developed in this study was applied to these two different types of data set to test its potential and robustness when dealing with lattice surface topographies acquired over varying depths and lattice details.

Acknowledging the discrepancy in the measured lattice features between the CX* and LX* batches, the direct comparison of the number of detected particles between the two groups was avoided.

7.2 Results of the particle detection and characterization of particles on the lattice surfaces

The protocol developed in this study to detect, measure and characterise the partially melted particles (PMPs) was tested on twelve surface topographies acquired by FVM on the lattices fabricated with the conditions CX* (C1-C6) and LX* (L1, L2, L4, L5, L6 and L8). In this section, the results of the particle analysis are presented, whilst the discussion on the robustness and the limitations of the method can be found in the following sections. Particles were successfully detected on all the analysed surface topographies (lattices CX* and LX*) and were measured in Mountains® in terms of length, width, minimum and maximum diameter. The detected objects were characterised by a projected area lower than 0.02148 mm² and were classed into SS, SM and SL groups depending on their length. The SS included particles with lengths from ~3 µm to 60 µm; SM included particles with lengths from 60 µm to 120 µm; SL included particles with lengths above 120 µm. As already mentioned in the Materials and Methods chapter, the range of these classes (SS, SM and SL) was defined based on the quality of the Zr702 powder used in the L-PBF lattice manufacturing, given that the shape and size of PMPs strongly depend on the characteristics of the material [139]. A detailed discussion on this choice will be presented in the following section. Briefly, as PMPs retain the size and shape of the metallic powder,

SS was defined to contain single particles with lengths lower than 60 μm , which corresponded to the 99% of cumulative distribution of the Zr702 powder. SM and SL were defined to contain larger particles and would likely contain particle clusters and solidified molten droplets from the spatter that typically occurs in the L-PBF [139].

Table 25 Results of the particle analysis for lattices fabricated with conditions CX. The minimum and maximum diameters, the length and width of detected particles are presented by average value and standard deviation. The data are presented by lattice condition and by particle class (SS, SM, and SL). Number of particles is the total number of particles detected with the protocol defined in the methods over three areas of 1mm² each. RD stands for Relative Distribution (%) and is the percentage of particles of a specific particle class over the total detected particles for a specific lattice.*

Lattice	Particle class	Number of particles	RD (%)	Minimum diameter (μm)	Maximum diameter (μm)	Length (μm)	Width (μm)
C1	SS	237	81	18.14 $\pm$ 6.96	33.35 $\pm$ 12.36	34.14 $\pm$ 12.72	21.98 $\pm$ 8.84
	SM	43	15	33.12 $\pm$ 11.30	79.45 $\pm$ 14.32	81.50 $\pm$ 14.53	45.75 $\pm$ 13.08
	SL	11	4	54.42 $\pm$ 18.45	151.30 $\pm$ 34.38	161.31 $\pm$ 34.74	78.62 $\pm$ 24.76
C2	SS	319	77	19.99 $\pm$ 7.47	36.14 $\pm$ 13.15	36.80 $\pm$ 13.47	24.80 $\pm$ 9.83
	SM	81	19	35.19 $\pm$ 12.32	80.98 $\pm$ 13.22	84.11 $\pm$ 13.93	48.49 $\pm$ 16.03
	SL	16	4	53.16 $\pm$ 21.24	148.19 $\pm$ 29.81	156.17 $\pm$ 34.67	86.19 $\pm$ 33.02
C3	SS	482	72	18.55 $\pm$ 8.14	33.76 $\pm$ 14.11	34.27 $\pm$ 14.50	22.68 $\pm$ 10.51
	SM	139	21	36.44 $\pm$ 11.58	81.67 $\pm$ 14.84	84.54 $\pm$ 14.97	51.51 $\pm$ 14.22
	SL	49	7	56.24 $\pm$ 24.29	154.80 $\pm$ 33.54	160.51 $\pm$ 34.50	87.87 $\pm$ 30.09
C4	SS	300	72	18.84 $\pm$ 8.39	35.14 $\pm$ 14.20	35.96 $\pm$ 14.78	23.23 $\pm$ 10.35
	SM	87	21	33.97 $\pm$ 12.68	82.76 $\pm$ 15.28	84.39 $\pm$ 14.85	46.37 $\pm$ 14.88
	SL	27	7	58.97 $\pm$ 20.74	151.83 $\pm$ 34.08	157.98 $\pm$ 33.66	88.09 $\pm$ 29.05
C5	SS	318	74	20.41 $\pm$ 8.37	36.12 $\pm$ 14.20	36.75 $\pm$ 14.70	24.86 $\pm$ 10.53
	SM	90	21	37.29 $\pm$ 11.26	80.63 $\pm$ 14.31	83.86 $\pm$ 14.69	51.80 $\pm$ 14.67
	SL	23	5	64.71 $\pm$ 23.27	158.65 $\pm$ 38.01	165.57 $\pm$ 43.03	87.10 $\pm$ 27.80
C6	SS	411	83	17.85 $\pm$ 7.39	35.08 $\pm$ 18.69	32.78 $\pm$ 13.33	21.76 $\pm$ 9.44
	SM	65	13	36.73 $\pm$ 13.44	74.03 $\pm$ 33.66	83.73 $\pm$ 17.59	51.15 $\pm$ 16.20
	SL	19	4	54.39 $\pm$ 21.73	105.36 $\pm$ 44.28	145.02 $\pm$ 20.14	82.31 $\pm$ 25.15

Table 25 and Table 26 summarise the results of the particle analysis relative to the lattices CX* and LX*, respectively: they report the number of particles – in each class – that were detected on the surface of the lattices, the relative distribution (RD, %) of each particle class over the total number of particles, and the sizes of the particles (length, width, minimum and maximum diameter) expressed by average value and standard deviation.

As it can be noticed from Table 25 and Table 26, only the detected particles of the SS class resemble to circular particles, with minimum and maximum diameters that are within the same range. For the other two classes (SM and SL), instead, the minimum and maximum diameters are statistically different, so referring to the dimensions of circular objects for these particles appeared inappropriate in this case. For this reason, the length and width of particles were finally chosen to characterise the size of PMPs.

Table 26 Results of the particle analysis for lattices fabricated with conditions LX. The minimum and maximum diameters, the length and width of detected particles are presented by average value and standard deviation. The data are presented by lattice condition and by particle class (SS, SM, and SL). Number of particles is the total number of particles detected with the protocol defined in the methods over three areas of 1mm² each. RD stands for Relative Distribution (%) and is the percentage of particles of a specific particle class over the total detected particles for a specific lattice.*

Lattice	Particle class	Number of particles	RD (%)	Minimum diameter (µm)	Maximum diameter (µm)	Length (µm)	Width (µm)
L1	SS	275	96.83	9.81±5.63	18.25±11.45	18.42±11.50	11.54±7.28
	SM	9	3.17	22.23±9.40	76.04±15.26	79.47±15.59	29.77±6.84
	SL	0	0.00				
L2	SS	301	96.78	9.73±4.98	19.76±12.20	20.12±12.54	11.62±6.42
	SM	10	3.22	31.30±5.032	75.26±13.93	78.32±12.78	39.15±7.61
	SL	0	0.00				
L4	SS	360	96.00	10.05±5.23	19.28±11.15	19.59±11.46	12.11±6.89
	SM	13	3.47	32.65±11.82	72.90±15.28	74.81±14.93	43.98±8.65
	SL	2	0.53	38.08±0.87	168.02±21.71	167.86±21.93	82.77±8.19
L5	SS	308	96.86	11.34±6.93	21.67±13.11	22.26±13.74	13.74±9.00
	SM	10	3.14	29.914±11.67	84.83±18.36	87.32±18.69	41.96±13.85
	SL	0	0.00				
L6	SS	200	100.00	6.50±4.43	13.36±11.24	13.67±11.60	7.78±6.01
	SM	0	0.00				
	SL	0	0.00				
L8	SS	306	97.14	10.30±5.20	20.50±12.11	20.96±12.681	12.29±6.83
	SM	9	2.86	26.64±13.21	75.42±12.79	81.24±12.74	38.64±14.69
	SL	0	0.00				

The length and width of the particles detected on the surface of lattices CX* and LX* are visualised in Figure 53a-b. As it can be noticed in Figure 53a-b, there is no significant

difference in the particle size (length and width) within any particle class (SS, SM, and SL) when CX* or LX* conditions are compared. For example, the SS particles – represented by the red circle in the two graphs – are characterised by similar lengths and width across the CX* group (or LX* group), regardless of the manufacturing conditions.

Overall, these results seem to indicate that the detected particles have similar geometry across the analysed surfaces, regardless of the specific manufacturing settings used to generate the surfaces. This aspect will be explored and discussed in section §7.5.

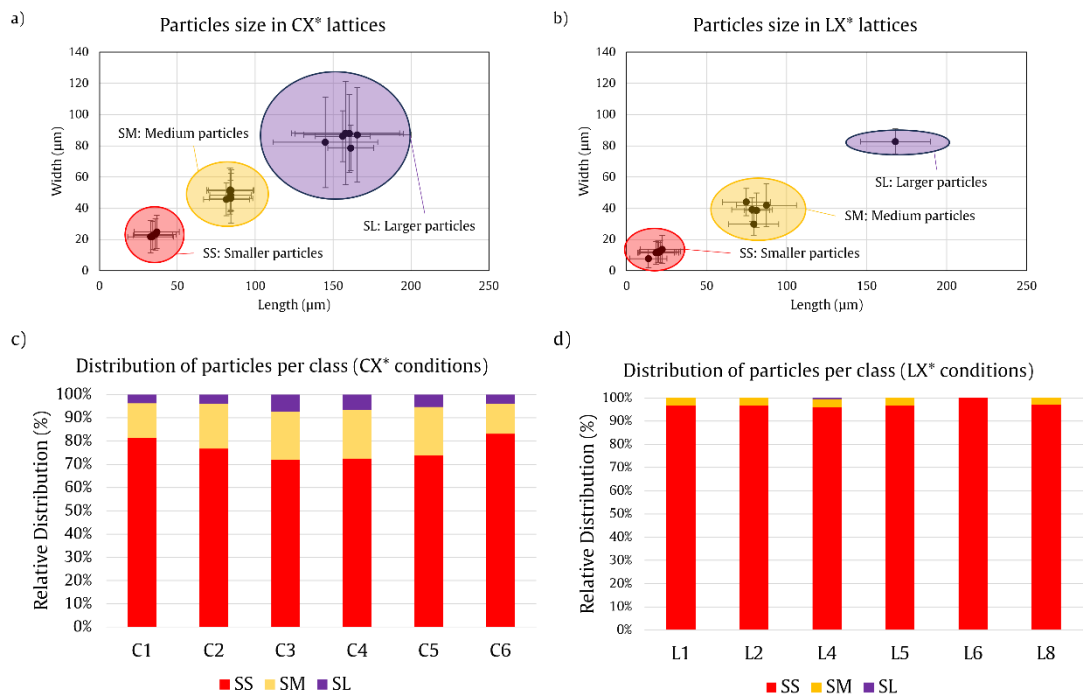


Figure 53 Particle size (length and width) of detected particles in lattices manufactured with conditions CX* (a) and LX* (b). The data are colour-coded by class: SS – red; SM – yellow; SL – purple; c) Relative distribution of detected particles (stacked by class) for the lattices CX*; d) Relative distribution of detected particles for the lattices LX*.

The relative distribution of the particles (detailed in Table 25 and Table 26) is graphically shown in Figure 53c-d for the particles detected on the surfaces generated with conditions CX* and LX*, respectively.

As shown in Figure 53c, the detected particles on the lattices CX* were distributed across the three classes (SS, SM and SL) with the same trend: the smallest particles (3-60 μm in length, SS class) was the greatest class, accounting for the 69 to 84% of all the detected

particles; the medium particles (60-120 μm in length, SM class) was the second biggest group, accounting for 13-24% of the detected particles; the biggest particles (length above 120 μm , SL class) was the smallest class, and accounted only for 1% to 7% of the detected particles.

The relative distribution of the particles detected in the lattices LX*, shown in Figure 53d, was characterised by a similar trend, although the SS class accounted for over the 90% of the detected particles and the SL class was empty or negligible, accounting for less than 1% (refer to Table 26).

7.3 On the proposed method for the detection of partially melted particles (PMPs) on lattices and on its robustness

The protocol developed in this study aimed at detecting the particles that adhere on the lattice surfaces during the manufacturing, also called partially melted particles (PMPs). PMPs are among the recognisable *features* on additively manufactured surfaces, and they could be recognised as part of the AM fingerprint on the surface topography. Although there is some interest [73] in characterising the PMPs as part of a topographical analysis in lattice structures (particularly for biomedical applications), there is still no available reported method that allows such analysis.

The method that was developed and tested in this study represents an attempt to analyse the surface topography of lattice structures under the perspective described above.

In this section, the protocol is discussed, addressing the main methodological choices and commenting them in relation to the literature. Then, the suitability of this method for the detection of PMPs is demonstrated, and the role of the PMPs geometry in the L-PBF process signature is discussed. Finally, the protocol's robustness is assessed, and the strengths and limitations of the methods are highlighted.

7.3.1 Discussion on the methodological choices for the particle detection and classification

This section comments the main methodological choices of the algorithm here developed and assessed and highlights the similarities and contrasts with the methods available in the literature.

The method proposed here is based on the feature-based characterization. As the additively manufactured lattice surfaces are “stochastic-deterministic” surfaces, they can be characterised by either field parameters (V-family) [152] or by features. However, the latter approach was preferred over the first for the following reasons. First and foremost, only feature-based characterisations (FBC) are able to detect, locate and characterise objects that populate the surface, such as the PMPs, which is the objective of the study. Second, FBC are more effective than field parameters in providing information on specific surface characteristics, when a significant deterministic content is present on the surfaces [152].

The developed method evaluates the lattice topography over three equally sized sample areas to allow quantitative comparison of detected particles.

The particle detection is performed without prior filtration of the topographical dataset. This choice was based on the considerations of Lou *et al.* [144], who observed that topographical segmentation can be applied without prior filtration when the PMPs (which normally considered as outliers on the topography) are the dominant features of the topography. This is particularly the case for the 45 degrees-inclined lattice struts [144] and the side surfaces of the horizontal struts, which is where the majority of the PMPs are expected.

The height pruning of 30 μm – which was selected to tune and filter the particle detection – was chosen in consideration of the layer thickness of the deposited powder during the L-PBF process. A similar pruning criterium (height pruning = 36 μm) was used by Lou *et al.* [144], although there the value was selected based on the standard deviation above the mean height of the surface. A trial with a height pruning of 20 μm had also been assessed

before finalising the selection of the pruning criterium, but this led to an over-segmentation of the PMPs into multiple semantically insignificant features (data not shown). Finally, the choice of setting the height pruning at 30 μm was preferred also in the light of the findings of Baccherwar *et al.* [147], who demonstrated that the surface roughness of upward L-PBF surfaces – which is directly affected by the amount of PMPs – is significantly affected by the thickness layer of the deposited powder.

The application of the edge detection leads to the segmentation of the whole area into particles. A representative example of a segmented topography and the relative detected particles is given in Figure 54. As it can be noticed from the segmented surface in Figure 54, the objects detected on the topography do not refer exclusively to PMPs, and they needed to be refined in order to capture only the particles of interest. It was then chosen to proceed with a first refinement, which was based on the particle's projected area. The ranges of the three classes (Big, Medium and Smaller) of particles were defined based on the qualitative and visual analysis of the whole segmented topography. The choice of the class limits is clarified in Figure 54 and is explained below.

The “Big” class (characterised by the biggest particle projected area, above 0.3996 mm^2) was defined to include the particles (more often one single particle) that corresponded to the connected horizontal struts and upper nodes of the upper exposed surface (see Figure 54). The “Medium” particles isolated relatively large smooth areas on 45-degrees oriented lattice struts. Indeed, Medium particles were absent in the LX* surface topographies (the corresponding areas were not measured, as illustrated in Figure 52). The “Smaller” class included potential PMPs (either single or in clusters). As shown in the histogram of Figure 54, this class was also the most numerous one.

After excluding the “Big” and “Medium” particles from the analysis, a second classification was defined in the attempt to identify the PMPs from other surface features. It is to be noted that the size of PMPs is variable [144] and unknown *a priori* [139]. With such uncertainty in the size of the objects of interest, the author concluded that a direct

size-based refinement of detected particles at the particle detection stage might have led to gross mistakes. Instead, it was chosen to proceed with a simple classification (not refinement) of the “Smaller” particles into three classes (SS, SM and SL) according to the particles’ length.

The proposed classification relies on the particle size of the metallic powder used in the manufacturing process, as described in the Materials and Methods chapter. The discussion of this aspect is reported in the following section (Section 7.3.2), which comments it in relation to the obtained results.

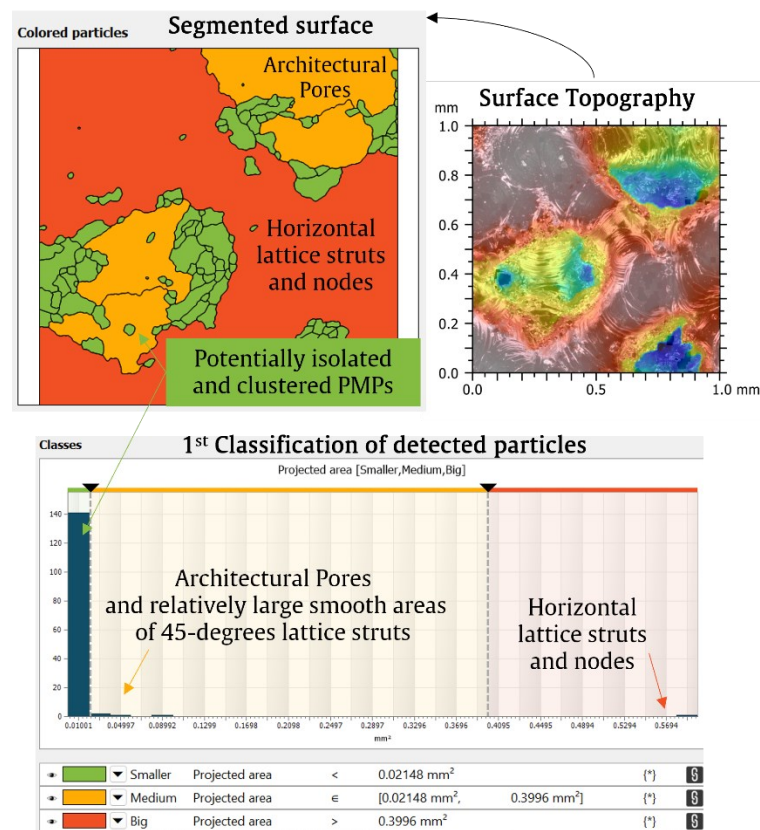


Figure 54 Illustration of the first refinement step in the detected particles, which was based on their projected area as shown in the reported table. The “Smaller” particles are the biggest class and potentially contains isolated and clustered PMPs; the “Medium” particles corresponded to areas over the architectural pores, that is on areas of the re-entrant 45-degrees inclined lattice struts; the “Big” particles (typically only one) corresponded to the upper exposed surface (horizontal lattice struts and nodes); Therefore, the “Big” and “Medium” particles were excluded from the analysis.

7.3.2 On the suitability of the developed method for the detection of PMPs

In this section, the SS, SM and SL classification is commented in the light of the obtained results, and the method developed here is discussed with respect to its ability to detect PMPs on L-PBF-manufactured lattice structures.

Figure 55a shows the distribution of the detected “Smaller” particles and the relationship of the three subsets SS, SM and SL with the source powder.

By comparing the two distributions (detected particles and Zr702 powder used for the manufacturing), it appears that:

- 1) the majority of the detected particles have dimensions similar to the original powder, with most of the particles being classed into SS and SM groups (this has already been shown in Section 7.2, with the particle distribution analysis)
- 2) the distribution of the “Smaller particles” is skewed to the left and its shape resembles to the one of the source powder.

As mentioned in the previous section, the PMPs’ size is variable and generally unknown *a priori* [139, 144]. However, it is acknowledged that PMPs that originate from the adhesion of single powder particles to the adjacent molten material during the manufacturing maintain the geometry (in shape and diameter) of the powder [139]. In a way, the geometry of these PMPs is a reflection of the source material that is employed for the manufacturing.

Most of the objects detected with the method here developed (belonging to SS and SM class) were compatible with this kind of PMPs. The hypothesis that SS and SM particles are actually relevant to single PMPs was tested analysing the lattice surface topographies with the superimposed detected particles. As shown in Figure 55b, the SS and SM particles are indeed compatible with the PMPs, whilst the detected SL particles do not appear to characterise any recognisable topographical feature.

These observations demonstrated that the method is suitable to detect PMPs on the surface of L-PBF lattice structures and suggested that particles with lengths that are

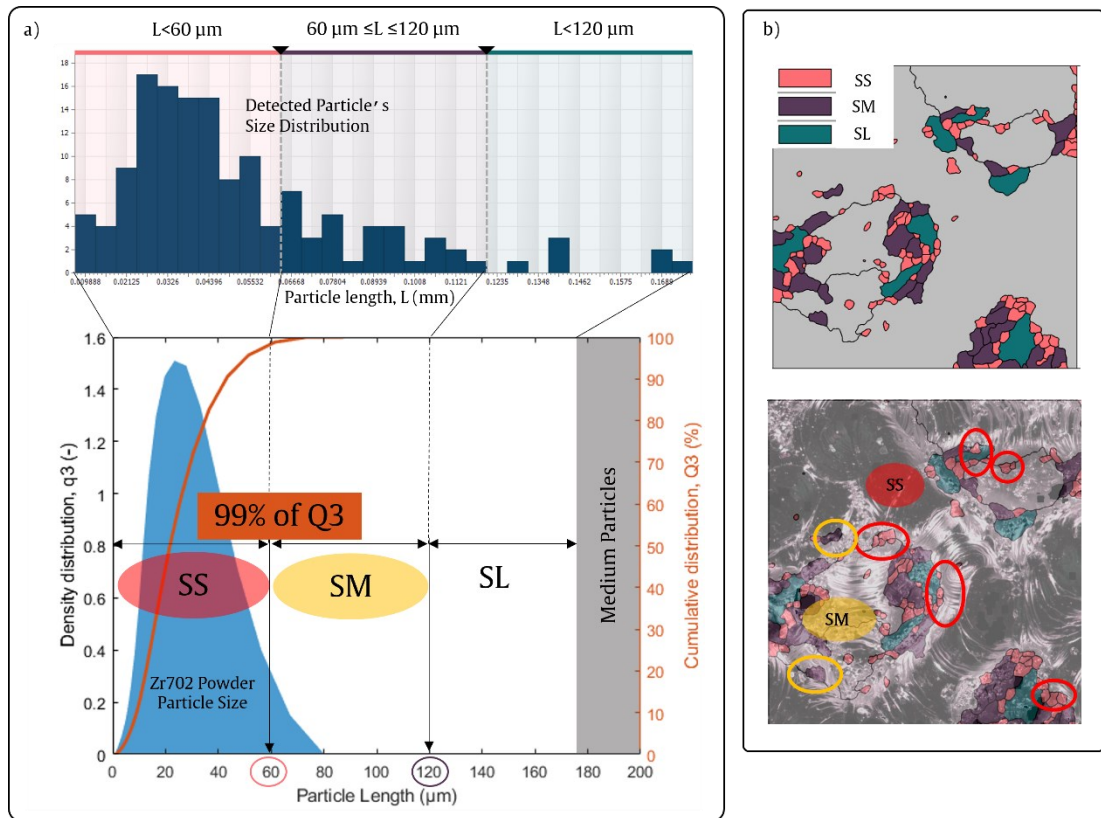


Figure 55 a) Classification of refined particles into the SS, SM and SL classes, according to the particles' lengths. The ranges of the classes were based on the length that corresponds to the 99% of cumulative distribution (Q3) of the powder particle size, which is $60 \mu\text{m}$. As the histogram above shows, the distribution of detected particles (contained in the SS, SM and SL groups) is skewed to the left and the majority of the detected particles has dimensions similar to those of the Zr702 powder. b) The original surface topography of a sample area ($1 \text{ mm} \times 1 \text{ mm}$) and the superimposed detected particles. From the visual analysis, it can be noted that only particles included in the SS and SM classes are compatible with PMPs.

beyond twice the of Q3-99% of the source powder (the current SL class) can be excluded *a priori* from the particle detection.

7.3.3 The robustness of the detection method

In order to assess the method's robustness, the areas over which the particle detection was performed were analysed in Fiji (ImageJ) and undetected particles were counted manually, as shown in Figure 56. The efficiency of the particle detection was assessed (with Equation 13) by comparing the number of undetected particles with the total number of particles (detected and undetected particles). The efficiency of the particle detection is reported in Table 27, and ranged between 84% and 96% for the CX lattice surfaces, and between 84% and 93% for the LX lattice surfaces.

Table 27 Efficiency of the proposed particle detection method developed within Mountains®.

Lattice	Detected particles	Undetected particles (visual analysis in Fiji)	Efficiency (%)
C1	291	54	84%
C2	416	73	85%
C3	670	28	96%
C4	414	27	94%
C5	431	39	92%
C6	495	36	93%
L1	284	54	84%
L2	311	45	87%
L4	375	28	93%
L5	318	26	92 %
L6	200	26	89%
L8	315	31	91%

Therefore, the method demonstrated similar efficiency on both the surface topographies that captured the full exposed surfaces (in this case represented by the CX* lattices), and on the surface topographies that captured the upper exposed surface only (LX* lattices case). It can be concluded that the method developed here offers the same rate of success in the particle detection, irrespective of the lattice components on which the particle analysis is performed.

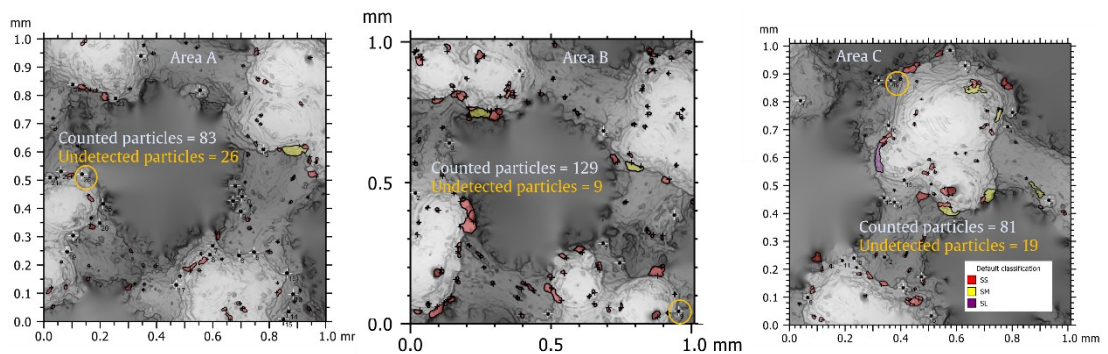


Figure 56 Example of particle counting on the sample L1 over the three areas (A, B, and C), in which the undetected particles have been counted manually.

A few superficial pores were also detected in the particle analysis and could be identified on the upper horizontal struts and nodes. These were typically classed in the SS group (see Figure 57). The pore “pick-up” issue in the detection was expected, given the nature of

the edge detection method, which is not able to discriminate between protruding and concave features. Indeed, the identification of pores on the analysed surfaces was possible only by visually comparing the segmented particles with the 3D view of the surface topography (refer to Figure 57).

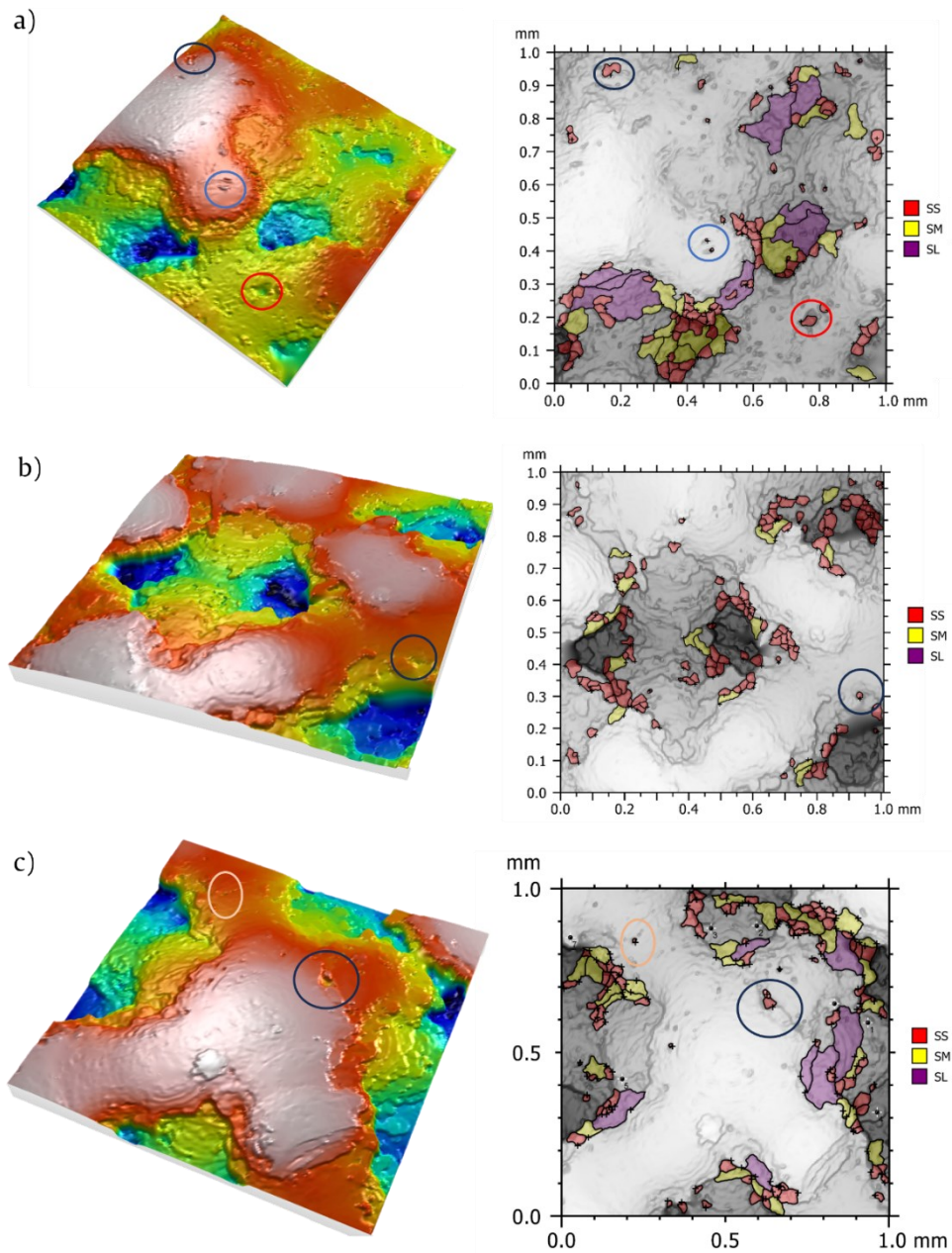


Figure 57 Representative examples of superficial pores that were wrongly detected as particles (in colour-coded circles) by using the protocol developed in this study. The pores could be identified by comparing the results of the particle analysis (on the right) with the 3D view of the same areas (on the left).

The pores detected here are at least 30 μm deep from the lattice surface, which means that these pores reveal the layer underneath (the powder layer is also 30 μm thick). The same kind of pores were also found by Thompson *et al.* [151] and Lou *et al.* [144], who investigated flat L-PBF surface topographies.

The fraction of these over the detected particles is negligible (refer to Figure 57 as an example), and therefore the method is suitable to characterise PMPs on lattice structures with porosities greater than 50%, when the probability of detecting a superficial pore is relatively low.

7.4 Characterising L-PBF lattice surfaces by particle density

Within this section, the author presents the particle density relative to the tested lattice surfaces and investigates its potential to capture the severity of the PMPs adhering phenomenon.

The particle density (particles/ mm^2) describes the number of particles that adheres on a surface and was defined in Equation 14 (Chapter 4, Materials and Methods).

In the light of the results obtained from the particle detection, which demonstrated that only SS and SM particles are compatible with PMPs, the author chose to define two manifold particle density parameters that are based on these features (SS and SM particles), rather than on all the detected particles. Therefore, the particle density of smaller particles and medium particles - $D_p(\text{SS})$ and $D_p(\text{SM})$, respectively – were calculated by adapting Equation 14. The number of SS detected particles (for both the CX* and LX* groups) and the particle density of smaller particles are shown in Table 28. The data relative to the SM particles can be found in Table 29. The results are graphically presented in Figure 58 for an easier fruition.

The lowest $D_p(\text{SS})$ for the CX* group was found on the lattice manufactured with condition C1 (with a $D_p(\text{SS}) = 79 \pm 13$ particles/ mm^2), whilst the maximum was recorded on the C6 lattice surfaces (with a $D_p(\text{SS}) = 137 \pm 15$ particles/ mm^2).

The lowest $D_p(SS)$ for the LX^* group was found on the lattice manufactured with condition L6 (with a $D_p(SS) = 67 \pm 9$ particles/mm²), whilst the maximum was recorded on the L4 lattice surfaces (with a $D_p(SS) = 120 \pm 21$ particles/mm²). With respect to the particle density referred to the medium particles $D_p(SM)$, the highest value was found on the C3-manufactured lattices, with a $D_p(SM)$ of 46 ± 3 particles/mm².

Table 28 Number of SS particles detected and the relative particle density $D_p(SS)$ for the CX and LX groups. A, B, C refer to the sample area particles were detected upon. Minimum and maximum values are coloured in green and red, respectively.

	Number of SS detected particles				Particle density of SS particles, $D_p(SS)$, (particles/ mm ²)			
	A	B	C	Total	$D_pSS(A)$	$D_pSS(B)$	$D_pSS(C)$	D_pSS (mean $\pm$ SD)
C1	97	73	67	237	97	73	67	79 $\pm$ 13
C2	100	126	93	319	100	126	93	106 $\pm$ 14
C3	188	146	148	482	188	146	148	161 $\pm$ 19
C4	99	88	113	300	99	88	113	100 $\pm$ 10
C5	92	94	132	318	92	94	132	106 $\pm$ 18
C6	134	120	157	411	134	120	157	137 $\pm$ 15
L1	82	117	77	276	82	117	77	92 $\pm$ 18
L2	75	100	126	301	75	100	126	100 $\pm$ 21
L4	126	123	111	360	126	123	111	120 $\pm$ 6
L5	90	112	106	308	90	112	106	103 $\pm$ 9
L6	66	78	56	200	66	78	56	67 $\pm$ 9
L8	136	90	80	306	136	90	80	102 $\pm$ 24

Table 29 Number of SM particles detected and the relative particle density $D_p(SM)$ for the CX and LX* groups. A, B, C refer to the sample area particles were detected upon. Maxima and minima values are coloured in red and green, respectively.*

	Number of SM detected particles				Particle density of SS particles, $D_p(SM)$, (particles/ mm ²)			
	A	B	C	Total	$D_pSM(A)$	$D_pSM(B)$	$D_pSM(C)$	D_pSM (mean $\pm$ SD)
C1	27	1	15	43	27	1	15	14 $\pm$ 11
C2	23	16	42	81	23	16	42	27 $\pm$ 11
C3	44	51	44	139	44	51	44	46 $\pm$ 3
C4	35	25	27	87	35	25	27	29 $\pm$ 4
C5	20	31	39	90	20	31	39	30 $\pm$ 8
C6	19	11	35	65	19	11	35	22 $\pm$ 10
L1	1	2	5	8	1	2	5	3 $\pm$ 2
L2	3	1	6	10	3	1	6	3 $\pm$ 2
L4	3	1	9	13	3	1	9	4 $\pm$ 3
L5	4	4	2	10	4	4	2	3 $\pm$ 1
L6	0	0	0	0	0	0	0	0 $\pm$ 0
L8	3	2	4	9	3	2	4	3 $\pm$ 1

The minimum value of SM particle density within the CX* group was 14 ± 11 particles/mm², relative to the C1 condition. With regard to the LX* group, the maximum particle density was 4 ± 3 particles/mm² (L4 condition) whilst the minimum particle density was recorded on the L6-manufactured lattice, where no SM particles were detected. Interestingly, the same manufacturing conditions (C3 and L4, for the CX* group and LX* group, respectively) led to the maximum values for both D_p(SS) and D_p(SM).

The same situation was observed when analysing the minimum D_p(SS) and D_p(SM): C1 and L6 conditions led to the minimum SS and SM particle densities in the CX* group and LX* group, respectively. Among the CX* group, C3 showed significantly higher D_p(SS) than C1, C2, C4 and C5 conditions; also, C6 was characterized by a higher D_p(SS) than C1 and C4. With respect to D_p(SM), C3 exhibited significantly higher particle density compared to all the other CX*-manufactured lattices. Interestingly, the same was found with respect to D_p(SM).

Among the LX* group, L6 was characterized by significantly lower D_p(SS) and D_p(SM) than all the other LX*-manufactured lattices.

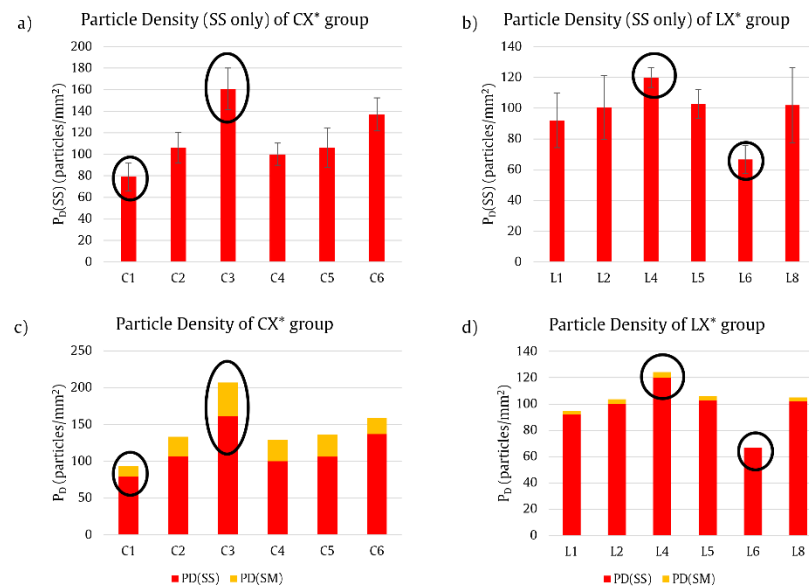


Figure 58 Results of the surface characterisation by particle density. above, the particle density of SS particles for the CX* group (a) and LX* group (b). The data are represented by the average value and the standard deviation. On the bottom, the stacked bar charts representing the mean particle density of SS and SM particles relative to the CX* group (c) and LX* group (d). The maxima and minima values are circled.

The characterisation of L-PBF manufactured lattice surfaces by particle density allowed to describe the severity of the PMP adhesion phenomenon and to capture the quality of these surfaces with a quantitative descriptor (D_{pSS} and D_{pSM} are expressed in particles/mm²). The PMPs density (expressed by D_{pSS} and D_{pSM}) leads itself well to a two-fold process-application topographical characterisation: on the one hand, the concentration of PMPs on the surface might be the topographical reflection of specific manufacturing settings of the L-PBF technology (process' fingerprint); on the other hand, it might be used to predict some performance of orthopaedic implants.

In this study, it was observed that D_{pSS} and D_{pSM} have the potential to discriminate between lattices fabricated with different settings and – therefore – they can be exploited to identify the manufacturing conditions used to generate their surfaces. As an example, here it was possible to recognise the lattice fabricated with L6 condition and distinguish it from the other LX* conditions because it bore the minimum particle densities D_{pSS} and D_{pSM} , and the difference of particle densities with the other LX* lattices was significant. This suggests that the D_{pSS} and D_{pSM} can be used to express (a part of) the *manufacturing signature* on the surface. This demonstrates that a set of only two *feature density parameters* might be able to (or at least contribute to) describe the topographical effect of specific L-PBF process parameters.

The characterisation through D_{pSS} and D_{pSM} could be used to characterise L-PBF lattices in the as-built and post-processed conditions. It is expected that treated lattice structures would exhibit a lower number of detected particles and a lower number of SS particles, in particular, as these are the first features that are attacked and removed in finishing processes (refer to Figure 59).

The same method can serve as a quality control tool of lattice surface topography after the manufacturing, for the assessment of the efficacy of surface treatments in the R&D¹⁴ of

¹⁴ In the development of surface finishing processes of lattices, it is critical to define the optimal process parameters that allow for the topographical improvement. In the case of L-PBF-manufactured lattices, the post-

surface finishing processes of lattices, and for characterising and comparing these complex surfaces through quantitative parameters (rather than by qualitative descriptions). Although surface-finished lattice surface topographies were not tested in this study, a trial was performed on the EDM-cut lattice surface to demonstrate the potential of this method in characterising and discriminating as-built and surface-finished lattices. The results of the particle detection performed on the as-built lattice and its relative EDM-cut surface are shown in Figure 59.

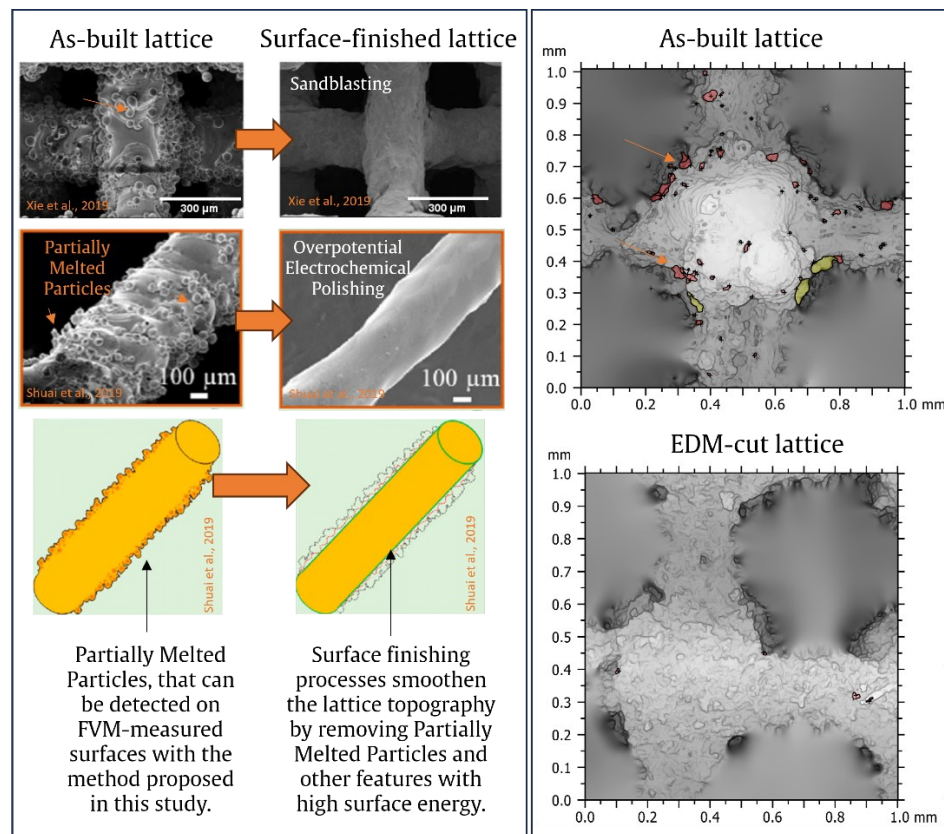


Figure 59 On the left, two examples of as-built and surface-finished lattice structures that show the dramatic difference in the quality of surface topography. On the bottom, the working principle of surface finishing treatments: the surfaces are smoothened by removing the material of high-surface energy features, such as the Partially Melted Particles. On the right, the results of the particle detection performed on the as-built lattice and an EDM-cut lattice, which demonstrate the suitability of discriminating as-built L-PBF surfaces from treated ones: PMPs were correctly detected on the first, whilst almost no particle was detected on the EDM-cut lattice. Images were adapted from [150, 156].

processing often involves chemical treatments, for which the time of reaction is of primary importance to remove material in excess (PMPs) without corroding excessively the lattice structure.

As it can be appreciated from Figure 59, the EDM-cut lattice upper surface was characterised by an irregular texture, generated by the electrical discharge in the cutting of the material, but did not exhibit any PMP. The particle detection on the EDM-cut surface was indeed close to zero, suggesting that surface-finished surfaces (which are likely to be much smoother than the tested EDM-cut lattices, as shown in Figure 59) would lead to a similar result. In conclusion, this study demonstrates the suitability of characterizing orthopaedic-aimed lattice structured by density PMPs and the potential of such topographical characterisation. The author would recommend characterising L-PBF surfaces by feature density, where the features should be selected based on the envisaged application.

Finally, the author hopes that the introduction of these topographical parameters and the demonstration of their potential for the assessment of L-PBF lattice surfaces will encourage other non-metrologists who deal with the same surfaces and/or orthopaedic-designed metallic surfaces to abandon meaningless¹⁵ profile or areal parameters (R_a , S_a , etc.). Instead, the author would incite them to choose surface parameters that are better suited to describe the special manufacturing process used in the surface generation (the L-PBF manufacturing's signature) and, at the same time, that represent an adequate link to a surface function/defect.

7.5 Partially melted particles as process' fingerprint on L-PBF lattice surfaces?

As demonstrated in the previous sections, the protocol developed in this study is able to detect partially-melted particles (PMPs) that constitute a recognisable feature of L-PBF-generated topographies. In this section, the link between the quality and quantity of the detected PMPs and the manufacturing process will be investigated, in order to explore the

¹⁵ Profile and areal parameters are meaningful when applied to surfaces that have specific characteristics. In the case of L-PBF surfaces and porous structures for orthopaedics, however, these parameters are hardly applicable (think of the difficulty in separating the roughness from the waviness on a free-form surface or on the lattice struts) and are not able to fully characterise the surfaces. In this context, profile and areal parameters are, therefore, meaningless.

potential of using PMPs as *L-PBF fingerprint* to gather information about the manufacturing process.

As shown in section 8.2, the PMPs exhibited similar geometry across the tested topographies. Indeed, the relative distribution of PMPs (Figure 53c-d) followed the same trend, regardless of the manufacturing conditions applied. These findings suggest that the PMPs distribution is not affected by the process parameters of L-PBF that were varied in the test (laser power, scanning speed, hatch spacing, contour distance). Therefore, the PMPs geometry is not revealing of the L-PBF process parameters. However, it was observed that the PMPs size distribution (particularly for the SS class) was similar to the source material used in the lattice fabrication, and that SS and SM particles were detecting single PMPs, which maintain the particle geometry (refer to Section 8.3.2 for a thorough discussion). Following these considerations, the author's educated guess is that the geometry of SS particles represents the *source powder's signature* on the surface topography of L-PBF (lattice) surfaces. Further research that investigates the PMPs on lattice structures generated by using powder that exhibit varying particle size distribution is needed to corroborate this hypothesis.

The manufacturing settings might be revealed by the quantity (or concentration) of PMPs on the surface. In Section 8.4, it was noted that the particle density parameters D_{pSS} and D_{pSM} varied between the lattices fabricated with different process settings (laser power, scanning speed, and hatch spacing, contour distance). Therefore, manufacturing conditions might be discriminated and identified through the D_{pSS} and D_{pSM} of PMPs on the L-PBF generated lattice surface topographies. In this sense, the D_{pSS} and D_{pSM} can be used to express (a part of) the *manufacturing signature* on the surface.

In this study, it was possible to recognise the lattice fabricated with a particular set of process parameters through the minimum particle densities D_{pSS} and D_{pSM} . However, the particle density parameters did not show a clear trend with varying process parameters and conditions, and further research is needed to understand which process parameters

affect the resulting amount of partially melted particles on the surface and the mechanism with which they influence the PMPs formation.

7.6 Conclusions and recommendations

The method to characterise the lattices' surface topography developed in this study is able to detect and characterise the geometry of partially melted particles (PMPs) that are generated by the L-PBF AM process. Therefore, the method is able to identify, measure and characterise a critical part of the L-PBF process's fingerprint.

The method offers a relatively high stability over a variety of manufacturing strategies (island and contour strategies for CX* and LX* groups, respectively) and process parameters (laser power, scanning speed, hatch spacing, contour distance), which makes the method rather reliable over the tested conditions and potentially applicable to other additively manufactured lattice structures with similar rates of success.

The proposed algorithm is based on considerations about the manufacturing and material used to generate the analysed surfaces, with the particle detection and characterisation being linked to physical parameters that are proven to affect the surface topography. The pruning criteria (a height of 30 μm) for the particle detection reflects the thickness of the powder layer in the L-PBF. The classification of detected particles is based on the source powder's particle size. Not only these methodological choices (relative to pruning criteria and particle classification) facilitate the interpretation of results, but also ease the generalisation of this method to other test cases and provide a readily adjustable criterium when the method is to be applied to additively manufactured lattices that are manufactured with different powders and consequently varying layer thickness.

It was observed that the detected particles that were compatible with the single PMPs belonged to the numerous class of SS and to the less populated SM class. The method here proposed can therefore be improved by filtering the detected particles by their length, excluding those with a length above the double of LQ3-99.

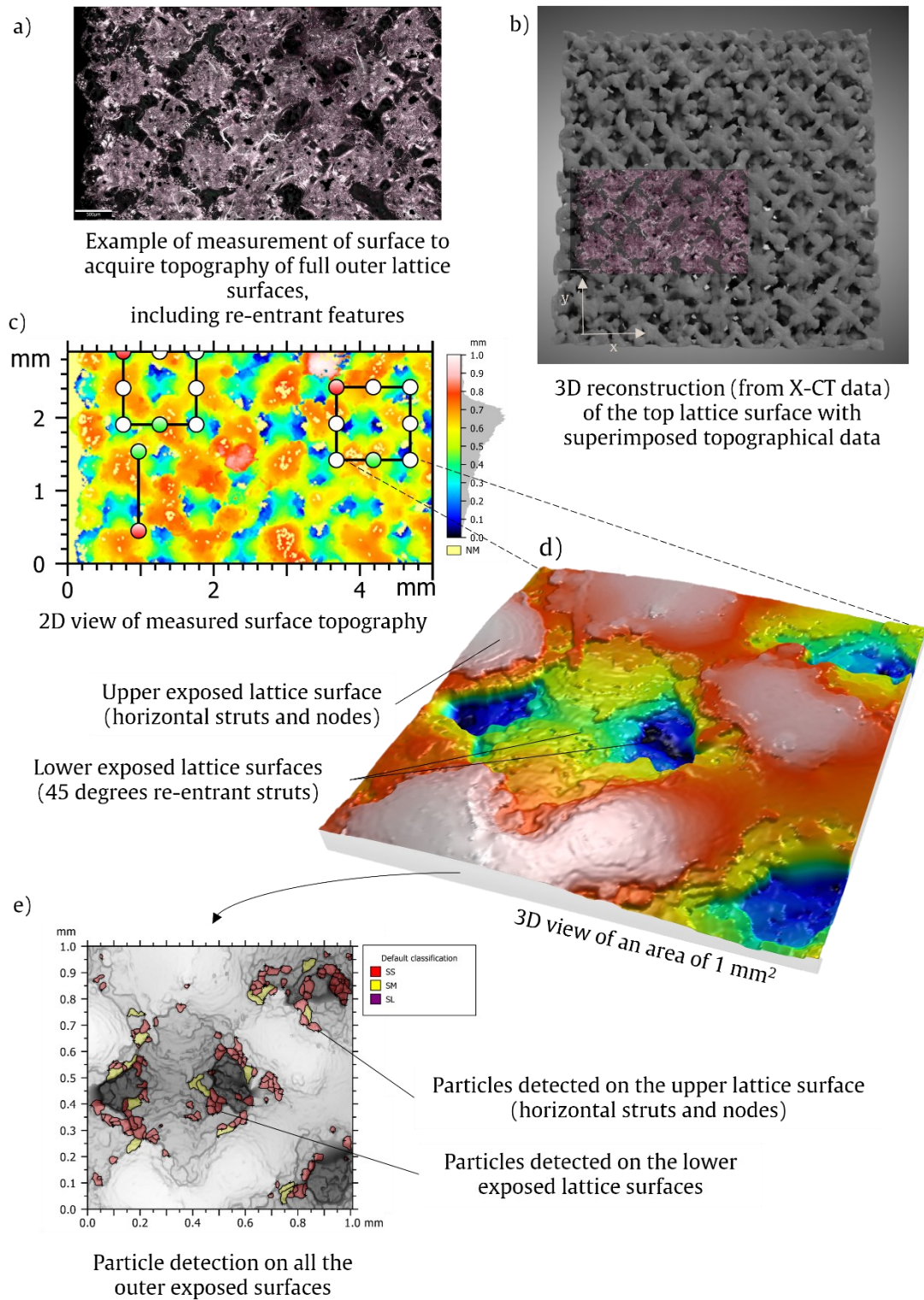


Figure 60 Recommendations on the measurement methods for further research.

The method has also the potential to recognise the process' fingerprint and to help distinguish surfaces generated with specific levels of process settings, and to then provide

indications on how to optimise the AM settings to engineer the surface topography during the manufacturing.

With respect to the quality of PMPs detected (in the SS and SM classes), it was observed that their geometry did not vary with the specific manufacturing settings used to generate the surfaces. Therefore, the geometry of PMPs did not seem to bear any process parameters' signature. Instead, it appeared to be the reflection of the source powder material employed in the L-PBF. Therefore, the author suggests treating the *geometry of PMPs* as the *source powder's signature* on the L-PBF lattice's topography.

A critical improvement of the detection algorithm would be represented by the introduction of 3D parameters in the edge detection algorithm within MountainsMap®, which would allow the exclusion of concave features (superficial pores) from the detected particles.

Finally, the author recommends measuring the surface topography of different lattices across the same length in Z direction (depth) and by keeping the starting measuring distance from the lattice constant. This would enable the acquisition of the surface topography with the same lattice features, that should include the “full exposed outer surface”, which coincides with the maximum surface area that is measurable by an optical instrument like the FVM. Figure 60 provides a visual guidance on the acquisition procedure for the surface topography of lattices and illustrates a successful particle detection over the entire exposed surface.

Chapter 8

Conclusions

This chapter presents the findings of the research work against the research questions and objectives set out in Chapter 1 and recommends directions for future work. Finally, it lists the dissemination work carried out during the PhD programme.

8.1 Research output

This thesis was driven by three research question, as outlined in Chapter 1:

1. *How can Zr702 be processed by L-PBF to fabricate octet lattice structures for an orthopaedic application?*
2. *How do L-PBF fabricated Zr702 octet lattice structures behave from a bio-mechanical perspective?*
3. *How can L-PBF-fabricated zirconium octet lattice structures be characterised in terms of geometry and surface topography?*

The answers to these questions constitute the conclusions of the PhD research work, and are given in the following subsections as outlined in Table 30.

Table 30 Structure of the conclusions (research output)

Research Question:	Conclusion given in subsection:
How can Zr702 be processed by L-PBF to fabricate octet lattice structures for an orthopaedic application?	§ 8.1.1 Optimal L-PBF process settings to fabricate Zirconium octet lattices with adequate geometrical accuracy and structural integrity
How do L-PBF fabricated Zr702 octet lattice structures behave from a bio-mechanical perspective?	§ 8.1.2 The mechanical and biological performance of zirconium octet lattices and the comparison with Zr702 bulk structures
How can L-PBF-fabricated zirconium octet lattice structures be characterised in terms of geometry and surface topography?	§ 8.1.3 A protocol to assess the geometry of L-PBF octet lattice structures with an “orthopaedic-tailored approach” § 8.1.4 A protocol for the characterisation of the surface topography of L-PBF zirconium octet lattice structures with an “orthopaedic-tailored approach”

8.1.1 Optimal L-PBF process settings to fabricate Zirconium octet lattices with adequate geometrical accuracy and structural integrity

The lack of previously published work on Zr702 on the fabrication of thin-walled structures (including lattice structures) and the awareness of the inherent inadequacy of bulk-tailored L-PBF process parameters for the printing of such objects motivated the experimental study on the L-PBF process optimisation with this material.

The author analysed the literature to define the conditions of “adequate geometrical accuracy and structural integrity” for orthopaedic-aimed lattice structures. These have been identified in the following: 1) an architectural porosity of at least 50%, no occluded architectural pores and a strut size as close as possible to the target design (geometrical accuracy condition); 2) a process-related porosity of 0.5% utmost (micro-structural integrity condition), 3) strut-to-node connectivity (material integrity through the lattice, or macro-structural integrity condition).

These conditions became the criteria for the (multi-objectives) L-PBF process optimisation throughout the study.

The L-PBF process optimisation studies were performed with two “printing strategies”: the “island strategy” and the “contour strategy”, which are commonly used in L-PBF.

With respect to the first, the investigated process parameters were the laser power (P), the scanning speed (v_s) and the hatch spacing (h_s). The factors were varied on three levels, and a Taguchi Design of Experiment was used to study a subset of the full factorial design (nine conditions out of twenty-seven).

In the second strategy, instead, the scanning speed (v_s) and the contour distance (CD) were varied on four and two levels, respectively.

The study allowed to identify the main factors that affect the geometrical accuracy and the structural integrity of the octet lattice structures, as well as to develop further the L-PBF process knowledge for Zr702 (notably in the form of process diagrams, which are available in Chapter 4).

The optimisation strategy developed by the author led to the identification of a set of parameters (Condition C2: $P = 150 \text{ W}$; $v_s = 1400 \text{ mm/s}$ and $h_s = 60 \mu\text{m}$) to be used with the “island strategy” to manufacture Zr702 octet lattices that are suitable for an orthopaedic application: they are characterised by an architectural porosity of 51%, a good material connectivity (macro-structural integrity) and a process-related porosity of $\sim 0.4\%$.

The lattices fabricated with the “contour strategy” ($P = 150 \text{ W}$; $v_s = 1400 \div 1800 \text{ mm/s}$; $CD = 50 \text{ or } 75 \mu\text{m}$) met all the criteria of the multi-objectives process optimisation. Therefore, the investigated conditions can be applied for the fabrication of orthopaedic-aimed Zr702 octet lattice structures. It is worth noting that the CD at $50 \mu\text{m}$ led to a significantly lower process-related porosity with respect to the optimal “island strategy” process settings ($0.12\text{-}0.16\%$ vs 0.4%). Nevertheless, no improvement in terms of geometrical accuracy was observed.

The author believes that better geometrical accuracy might be achieved by reducing the laser power (either in “island strategy” or “contour strategy”) to 100 W and would recommend investigating the effect of various printing strategies on the generation of the lattice struts and nodes, to develop new compensation strategies aimed at improving the geometric accuracy for L-PBF lattice structures.

8.1.2 The mechanical and biological performance of zirconium octet lattices and the comparison with Zr702 bulk structures

The mechanical and biological assessments of the Zr702 octet lattice structure were performed on the lattice fabricated with the optimal “island strategy” process settings (C2). Bulk Zr702 samples were bio-mechanically tested to assess the impact of the porosity on the various performances.

This study is the first work that is focused on the bio-mechanical assessment of porous Zr702 structures.

The lattice structures were mechanically tested under quasi-static compression test according to the ISO 13314 [93] and the elastic properties of the structure were assessed.

The elastic gradient was 5.64 ± 0.03 GPa, which is close to the lower limit of the elastic moduli of compact bones (5 ± 40 GPa) and meets the elastic modulus of the tibia (5.71 ± 1.27 GPa).

The mechanical tests also revealed that the structures fabricated by L-PBF with the C2 settings exhibit consistent mechanical behaviour both in the elastic region and in the plastic region, suggesting that a good control of the fabrication of the lattice structure was achieved during the manufacturing.

By comparing the lattice's elastic gradient with the Zr702's elastic modulus (94.75 GPa), it was found that the 51% architectural porosity of the lattice led to a reduction of 94% of the material's stiffness.

The Zr702 octet lattices fabricated and tested in this study exhibited a higher compressive proof strength (200 MPa) compared to Ti6Al4V octet lattices, demonstrating the superior yield strength of Zr702 over Ti6Al4V, and the higher load-bearing capacity of this material over the classic, gold-standard, orthopaedic material.

Zr702 octet lattice structures with porosities above 60% have the potential to match the less stiff *trabecular bones*, and would allow to cover the full range of bones' moduli (compact and trabecular bones). Further studies investigating the elastic gradient and compressive proof strength of Zr702 octet lattices with porosities higher than 51% are recommended.

Overall, the lattices exhibit mechanical properties that are relevant to an orthopaedic application. Their introduction in compact bone-substituting implants (particularly tibia) is expected to reduce the risk of stress shielding with respect to fully bulk metallic prostheses. Future work should focus on the fatigue testing of these lattice structures, to assess the long-term mechanical resistance of these L-PBF structures.

The biological performance of the lattice was evaluated in terms of cell viability (the number of living cells at a specific time of cell culture on a substrate), which was assessed

with two complementary tests (the MTT and the kit Live/Dead with fluorescence imaging). Tissue-culture plastic was used as a “control” substrate in both the biological tests. Briefly, the results indicate that the cell viability for osteoblast-like MG-63 cells is stable on flat ground Zr702 bulk surfaces, at least until 72 hours of cell culture (albeit lower compared with the tissue-culture plastic), confirming the good biocompatibility of the material. Furthermore, it was observed how the topographical grooves on the ground flat zirconium disks guided the cell adhesion and spreading onto the material’s surfaces. The cell viability on zirconium lattice appeared to be lower than zirconium bulk disks, especially after 72 hours of cell culture. Although the difference was not statistically significant, the author suspects that zirconium lattices do offer different biological performance with respect to the bulk material, due to the surface topography of the lattice features. Indeed, she would like to suggest that the cellular behaviour on the zirconium lattice depends on the particular lattice surface with which the cells come into contact with and that, in general, it differs from the cellular behaviour on the ground flat zirconium disks.

The author outlined three potential cellular behaviour/biological outcomes. The first, that is relevant to the cells that attach to the flat top ground surfaces of the lattice, suggests that MG-63 cells can adhere well to these surfaces and maintain their metabolic activity (hence live). The second, that is applicable to the internal features of the lattice, assumes that the complex, highly irregular, as-manufactured surface topography of lattice struts and nodes hinder a good cell adhesion, which in turn results in low metabolic activity for MG-63 cells, and leads to cell death. The third is relevant to the cells on the bottom of the lattice that were found dead: in this case, the author presumes that cells were killed by the lack of fresh media availability.

In summary, the author concludes that zirconium is indeed biocompatible; however, L-PBF lattice structures need to undergo a chemical (or electrochemical) treatment aimed at smoothening the internal surfaces, to maintain the material’s biocompatibility.

The author acknowledges the limitations of this experimental study in relation to the limited sample size and would like to invite others to proceed on the route here marked, using these results as a basis for further research. In this regard, the author would like to emphasise once again the importance of using two complementary assays in biological tests. This approach allowed, first, to check the quality of the experiments (for example, the agreement of the results obtained in the two parallel tests enabled to build confidence on the output data) and, second, to gain a better understanding of the cell behaviour on the various substrates, particularly for Zr702 in bulk and lattice, for which the statistical test concludes that there is no difference in terms of cellular behaviour. The possibility to locate and visualise the cells seeded onto the different substrates by fluorescence staining (kit Live/Dead), combined with the ability to count (indirectly) the living cells by MTT test, allowed to appreciate at once the cell viability, density and morphology on different areas of the various samples. It was indeed by adopting this approach that the author could suggest how the surface topography might have played a role in the cellular adhesion on bulk and lattice zirconium.

8.1.3 A protocol to assess the geometry of L-PBF octet lattice structures with an “orthopaedic-tailored approach”

The manufacturing of (octet) lattice structures via L-PBF is typically accompanied by a certain geometrical mismatch between the designed and fabricated lattice. Such discrepancy should be controlled to ensure the proper functioning of the lattice in orthopaedic implants.

At the current time, however, the geometry of lattice structures embedded in commercial orthopaedic prostheses are not normed, and few characterization protocols exist for the assessment of the lattice geometrical accuracy.

The author developed a protocol for the measurement of the geometry of L-PBF octet lattice structures with the aim of establishing whether the geometry of the fabricated lattice is adequate for an orthopaedic application.

The criteria for the geometrical accuracy – which were identified in the literature review on metallic lattices for orthopaedics, and that had guided also the L-PBF process optimisation (refer to §8.1.1) - were: 1) an architectural porosity of at least 50% (versus a target design of 65%); 2) no occluded architectural pores and 3) a strut size as close as possible to the target design (200 μm).

The L-PBF fabricated lattices were scanned by X-rays Computed Tomography (X-CT) to obtain 3D image reconstructions of the L-PBF lattices and to allow the full volumetric geometrical measurement and characterisation.

Finally, the algorithm developed within ORS Dragonfly allows to segment the X-CT images with a series of Boolean operations and to characterise the extent and quality of the architectural porosity and occluded architectural pores. As such, this procedure allows to establish whether the conditions 1) and 2) for the geometric accuracy are verified for a given lattice. Importantly, this algorithm can be used on any type of lattice topology.

The method developed in ORS Dragonfly also enabled for the extraction of the “lattice” Region of Interest (ROI), which was further analysed in MATLAB® with a series of originally developed algorithms.

The MATLAB® algorithms (which are reported in Appendix I) allow to automatically measure the diameters of struts and nodes of the octet lattices along the different orientations of interest. In particular, the method allows to measure the diameter of horizontal and diagonal (oriented at 45° from the building plane) struts, and the dimensions of the nodes in the horizontal and vertical planes. As such, it enables to assess the condition 3) for geometrical accuracy.

This method – which entailed the rotation of the 3D matrix of the segmented X-CT data along the directions of interest and the generation of 2D cross-sections that are orthogonal to such directions – represents the first protocol that enables for the automatic measurement and characterization of lattice features in more than one direction.

The method was validated on nine X-CT data sets, relative to the octet lattices fabricated with nine different manufacturing conditions.

The automatic algorithm developed in this study allows to fully characterise the geometry of the octet lattices and might be used for the assessment of Zr702 octet lattices in new L-PBF optimisation studies. The algorithm can be used as such to characterize the lattice topologies that exhibit horizontal and 45°-oriented struts, like centred-body cubes, Kelvin structures, etc. Furthermore, the MATLAB codes can be easily adapted to extract 2D images with the lattice cross-sections in other directions, enabling for the characterisation of different lattice topologies.

8.1.4 A protocol for the characterisation of the surface topography of L-PBF zirconium octet lattice structures with an “orthopaedic-tailored approach”

The surface topography plays a crucial role in determining several performances of a whole component, including the implantation outcome of orthopaedic prostheses. As such, the surface topography of L-PBF lattice structures of orthopaedic-aimed applications should favour (or at least not hinder) the osteointegration process.

However, the surface topography of L-PBF lattices is not currently measured, characterised, or assessed in industry. In fact, the surface topography of orthopaedic-aimed lattice structures is currently not normed by any regulatory body.

The literature review conducted at the beginning of the PhD programme allowed to identify several gaps and challenges in the surface characterisation of L-PBF lattice structures, and guided the author in developing a topographical characterisation protocol that would be relevant to L-PBF orthopaedic lattice structures.

Among the others, the difficult access to the lattice surfaces, the limited development of the topographical characterisation for additively manufactured surfaces, and the lack of established procedures for the measurement and characterisation of lattice structures have so far prevented the full topographical characterisation of L-PBF lattices. In addition to these, contradictory findings relative to biological studies and surface topography

(probably exacerbated by poor surface characterisation) still pose significant challenges in identifying the features that make a “good” orthopaedic surface.

Nevertheless, the scientific community seems rather unanimous in identifying the so-called “partially melted particles” (PMPs) – which are a typical L-PBF-generated surface topographical *feature* - as a cause of concern for the long-term biocompatibility of L-PBF-made prostheses.

Following the concerns raised on the presence of partially melted particles (PMPs) on the surface of L-PBF-manufactured orthopaedic implants, the author focused her efforts in detecting and characterising such *features* on the L-PBF-generated lattice topographies.

Therefore, an original method that allows for detection, measurement and characterisation of the PMPs was developed and tested on twelve L-PBF lattice structures. To the best of the author’s knowledge, this is the first study that applied the feature-based topographical analysis for the detection of PMPs to L-PBF lattice structures.

Briefly, the protocol developed in Mountains® to detect and characterise the PMPs on L-PBF lattice surfaces is based on the “feature-based characterisation” and can detect, locate and measure specific features that populate the surface topography.

The detection algorithm here proposed is based on the edge detection without prior data filtration, and a height pruning of 30 µm (the same as the powder layer thickness in the L-PBF), which allowed to segment (or label) correctly several L-PBF topographical features, including the PMPs. The segmented features must undergo a further refinement to capture only the PMPs, which are identified in the following classes:

SS: Projected area < 0.02148 mm² AND Length < 60 µm

SM: Projected area < 0.02148 mm² AND Length ∈ [60 – 120] µm

By testing the protocol on twelve lattice topographies generated with different manufacturing settings, it was found that the geometry of the detected PMPs (particularly the SS particle class) is a reflection of the particle size of the source powder used in the manufacturing. Moreover, the particle density of specific classes (D_{pSS} and D_{pSM}) can

discriminate the manufacturing conditions (process settings), although more research is needed to determine which process parameters are significant factors for the resulting D_{pSS} and D_{pSM} .

Overall, the findings suggest that the *PMPs' geometry* might be the *source powder's signature* on the L-PBF lattice structures, whilst the *PMPs' density* could represent the *manufacturing's fingerprint*. The author would like to stress the complementarity of the information obtained by characterising both the geometry and the number of PMPs on lattice surfaces.

The method shows similar efficiency of detection (robustness) of the lattice topographies acquired over different depths, hence on topographical data sets which capture different surface extensions and details. Nevertheless, the author recommends acquiring the surface across the full measurable area, to allow the complete surface characterization and a rigorous comparison between L-PBF lattice and porous structures, in general.

The characterisation method has the potential to discriminate not only between L-PBF lattice structures in their untreated form, but also to characterise and distinguish between as-fabricated and surface-finished lattice structures. In this sense, the proposed method can serve three purposes: 1) as a quality control tool to assess the lattice surface topography after the manufacturing in industry; 2) to assess the efficacy of surface treatments in the R&D of post-processes aimed at enhancing the surface texture; 3) to compare the quality of different L-PBF lattice structures in academic environments.

The author hopes this study can open the door to a new characterisation method – especially in the biomedical field – that is relevant to L-PBF lattice structures and that is truly informative of the quality of these surfaces.

8.2 Research Dissemination

Parts of the present work were presented at peer-reviewed international conferences:

1. EUROPM 2021, online

- Crocco, B., Imbrogno, D., Tamimi, S., Attallah, M., Butler, D., (2021). “Optimization of selective laser melting process for zirconium lattices as orthopaedic implants”.

2. MET&PROPS 2022 in Glasgow, UK

- Crocco, B., Imbrogno, D., Tamimi, S., Attallah, M., Butler, D., (2022). Characterisation of lattices for orthopaedic implants produced via Laser Powder Bed Fusion with X-rays Computed Tomography and Focus Variation Microscopy.

3. World Titanium Conference, Visit to the Advanced Forming Research Centre (AFRC), UK

- Crocco, B., Butler, D., Tamimi, S. (2023). Additive manufacturing of zirconium lattices for orthopaedic application.

Appendix I

MATLAB codes developed for data analysis

This appendix encloses the MATLAB codes that were developed by the author to perform 1) the geometrical characterisation of lattice structures by automatic image analysis from segmented X-CT data (§A.I.1-A.I.8); and 2) the data analysis relative to the compression test of the material Zirconium (§A.I.9).

The workflow of the algorithm for the geometrical characterisation of lattice struts and nodes was shown in Chapter 3 in Figure 19 Workflow of the algorithms developed in MATLAB and illustration of horizontal and diagonal struts, nodes on the XY plane and on the XZ plane.

A.I.1 BC_Script_v9_NODE_XY

```
% BC_Script_v9_NODE_XY performs the geometrical characterisation of lattice  
% nodes on the XY plane.
```

Loading of images

```
Img_FolderPath = 'C:\Users\beatr\OneDrive - University of  
Strathclyde\PhD\Documents\MATLAB'; %'C:\Users\prb19227\Documents\MATLAB'  
Img_LatticeName = 'L1-Lattice'; % 'L1-Lattice' 'L2_lattice' 'L3_Lattice' 'L4_Lattice'  
'L6_Lattice' 'L7_Lattice'  
Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);  
  
if ~isfolder(Img_FullFilePath) % the tilde '~' is a logical negation. So the if  
condition is true (the file exist), the algorithm goes to ELSE condition.  
    disp('Please update the file path')  
    return  
  
else  
    imds = imageDatastore(Img_FullFilePath,...  
        'IncludeSubfolders',true,'FileExtensions','.tiff','LabelSource','foldernames');  
end
```

scale management

```
Pixelwidth_micron = 7.24575; %(1 pixel = 7.24 micron)
```

Choose what should be computed (= 1 only)

```
Compute_BlobAtEdgeFiltering = 1; % Remove blobs in touch with the edge (circular  
shape only !!!)  
Compute_BlobSizeColour = 1; % Colour blobs according to their area  
Compute_BlobSizeFiltering = 1; % Filter blobs in touch with the outer circle
```

Reading the images

```
for i_img = 220:347  
  
    Img = readimage(imds, i_img); % reads the i-th image in the image store imds  
    Img_bw = Img == 255;% Convert image to binary  
    numberImg = string(i_img);
```

Remove blobs at edge

```
if Compute_BlobAtEdgeFiltering == 1  
    se_size = 25;  
    DispResult = 0; % Set 'DispResult = 1' if intermediate steps should be displayed  
    [Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img_bw, se_size, DispResult);  
    Img2Use = Img_NoEdge;  
else  
    Img2Use = Img_bw;  
end
```

Image properties

```
[ImgHeight_pixel, Imgwidth_pixel, ~] = size(Img2Use); % Size of the image Img_bw  
Img_Labeled = bwlabel(Img2Use, 8); % 8 : connectivity %bwlabel LABELS connected  
components in a binary image  
Properties = regionprops('table', Img_Labeled ,  
{ 'Area', 'ConvexHull', 'PixelList', 'EquivDiameter' }); % Changing 'all' by the selection  
of desired properties decrease computation time  
Properties.AreaMicron = Properties.Area .* ((Pixelwidth_micron)^2); % creates a  
column in the TABLE Properties called "AreaMicron"  
Properties.EquivDiameterMicron = Properties.EquivDiameter .* (Pixelwidth_micron);  
  
SliceNumber_imgI = zeros(height(Properties),1);  
SliceNumber_imgI(:) = i_img; %it's a vector  
Properties.SliceNumber = SliceNumber_imgI;
```

Segmentation - Filtering : keep only the struts of interest

```
if Compute_BlobSizeFiltering == 1  
  
    %--- Trigger definition  
    trig_low_Micron = 200000;  
    trig_high_Micron = 300000;
```

```

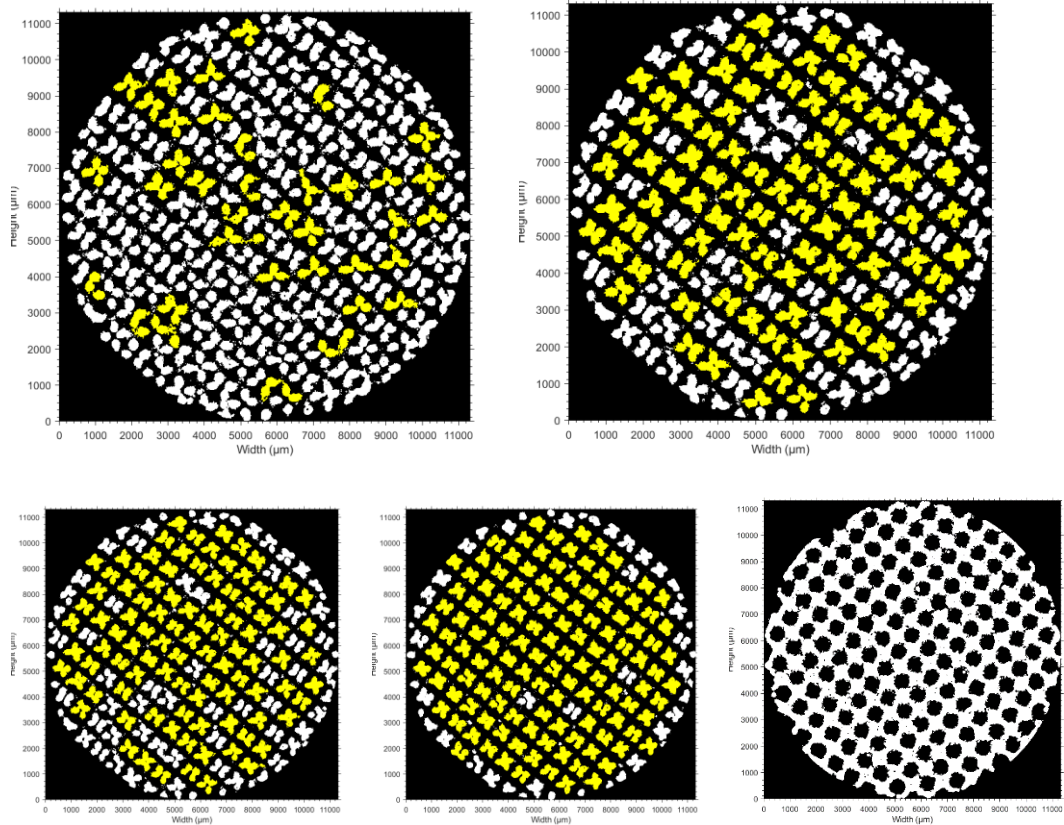
%--- Filtering : Identify the struts of interest (SOIs)
Index_SOI = find((Properties.AreaMicron >= trig_low_Micron) &
(Properties.AreaMicron <= trig_high_Micron)); % SOI = Strut Of Interest
Mask_SOI = ismember(Img_Labeled, Index_SOI); % Extract only those blobs that meet
our criteria
Boundaries_SOI = bwboundaries(Mask_SOI, 8, 'noholes'); % 8 : connectivity

%--- 1st step : Convert data ( $\mu\text{m}^2$ ) to create coloured image (+ Conversion pixel
=>  $\mu\text{m}$ )
[ColourBlob_Img, BinLimits, cmap] = Fctn_ColourBlob_Area(Img2Use,
Properties.AreaMicron);
% ColourBlob_Img : image
% BinLimits : max and min area values
% ColorMap : Matlab object permitting to manage image colours

%--- 2nd step : Create figure and axes, display image & define required settings
Fig_Strut = figure('NumberTitle', 'off', 'name', 'filtered struts and strut
measurement' + numberImg, 'Color', [1 1 1]); % Create figure
set(Fig_Strut, 'Units', 'normalized');
%   ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
%   set(Fig_Area, 'OuterPosition', ImgFigOutPosition); % Uncomment to define
figure position
Axes_SOI = axes(Fig_Strut); % Create axes
cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
hold(Axes_SOI, 'on')
%----- Add scale in  $\mu\text{m}$  to x & y axis
micron_Ticks_Step = 1000;
Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron, ImgHeight_pixel,
Imgwidth_pixel)

imgoverlay = imoverlay(Img_bw, Mask_SOI, 'yellow');
imshow(imgoverlay, 'Parent', Axes_SOI);
hold(Axes_SOI, 'on')
%----- Add scale in  $\mu\text{m}$  to x & y axis
micron_Ticks_Step = 1000;
Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron, ImgHeight_pixel,
Imgwidth_pixel)
hold(Axes_SOI, 'on')

```



```

%--- Filtering : Identify the struts of interest (SOIs)
Index_SOI = find((Properties.AreaMicron >= trig_low_Micron) &
(Properties.AreaMicron <= trig_high_Micron)); % SOI = Strut Of Interest
Mask_SOI = ismember(Img_Labeled, Index_SOI); % Extract only those blobs that meet
our criteria
% figure, imshow(Mask_SOI);
% imshow(Mask_SOI, [0 80]);
% hold(Axes_SOI, 'on')
%
% %----- Add scale in  $\mu\text{m}$  to x & y axis
% micron_Ticks_Step = 1000;
% Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron, ImgHeight_pixel,
Imgwidth_pixel)

%--- Storing SOIs properties in table
Props_SOI = Properties(Index_SOI, :); % Creates table with regionProps only for
the indexed SOIs

%--- Store the filtered struts in singular vectors and cells. To do this, it's
better
%to store the data in separated vector and then to recombine the table

SliceNumberV = Props_SOI.SliceNumber;
AreaMicronV = Props_SOI.AreaMicron;
ConvexHullC = Props_SOI.ConvexHull;
PixelListC = Props_SOI.PixelList;
EquivDiameterMicronV = Props_SOI.EquivDiameterMicron;

```

```

if i_img == 1
    i0 = 0;
    i = i0 +1;
    j = i + length(SliceNumberV) -1;

    SliceNumber(i:1:j,1) = SliceNumberV(:);
    AreaMicronTot(i:1:j,1) = AreaMicronV(:);
    ConvexHull(i:1:j,1) = ConvexHullC(:);
    PixelListTot(i:1:j,1) = PixelListC(:);
    EquivDiameterMicron(i:1:j,1) = EquivDiameterMicronV(:);
    i0 = j;
else
    i0 = j;
    i = i0 +1;
    j = i + length(SliceNumberV) -1;
    SliceNumber(i:1:j,1) = SliceNumberV(:);
    AreaMicronTot(i:1:j,1) = AreaMicronV(:);
    ConvexHull(i:1:j,1) = ConvexHullC(:);
    PixelListTot(i:1:j,1) = PixelListC(:);
    EquivDiameterMicron(i:1:j,1) = EquivDiameterMicronV(:);
    i0 = j;
end

%--- Making the table with all the struts
A = table(SliceNumber, AreaMicronTot, ConvexHull, PixelListTot,
EquivDiameterMicron);

end

end

```

Analysis of the regions with Ellipse Axes, Feret Diameters and Bounding box

<https://uk.mathworks.com/help/matlab/ref/waitbar.html> and modified

```

% MinorAxisLength (or MajorAxisLength) is the Length (in pixels) of the
% minor axis (or major axis) of the ellipse that has the same normalized
% second central moments as the region, returned as a scalar. Second
% Central Moments are IMAGE PROPERTIES often used after segmentation to
% help the analysis.
% Props_SOI.MajorAxisLengthMicron = Props_SOI.MajorAxisLength .* (Pixelwidth_micron);
%adding Minor and Major Axis of the Ellipse in micron
% Props_SOI.MinorAxisLengthMicron = Props_SOI.MinorAxisLength .* (Pixelwidth_micron);

P_SOI = table2struct(A); % creates a STRUCTURE
FeretP = feretPropertiesBEA5(P_SOI, Pixelwidth_micron);

% Equivalent diameter
Fig_Histo = figure('NumberTitle', 'off', 'name', 'Histogram Equivalent Diameter Node
xz(μm)', 'Color', [1 1 1]); % Create figure
histogram([FeretP.EquivDiameterMicron]);

Eq_D_avg = mean([FeretP.EquivDiameterMicron]);

```

```

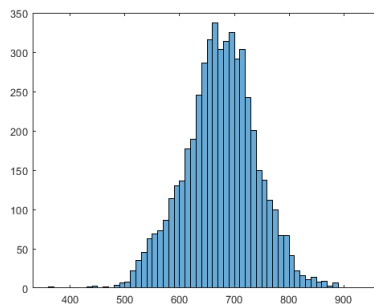
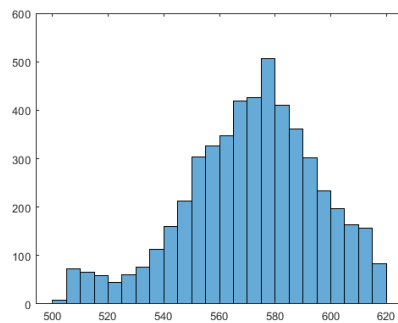
% Orthogonal diameter
Fig_HistoOF = figure('NumberTitle', 'off', 'name', 'Histogram Orthogonal Diameter
Node XZ ( $\mu$ m)', 'Color', [1 1 1]);
histogram([FeretP.OrthogonalDiameterMicron]);

Ortho_avg = mean([FeretP.OrthogonalDiameterMicron]);

% Summary Table ST
ST.DiagonalStrutsRelativeToLattice = convertCharsToStrings(Img_LatticeName);
ST.Equivalent_Diameter_micron = Eq_D_avg;
ST.EquivDiamtMicron_SD = std([FeretP.EquivDiameterMicron]);
ST.OrthoDMicron = Ortho_avg;
ST.OrthoDM_SD = std([FeretP.OrthogonalDiameterMicron]);
ST.MaxFeretDiameter_micron = mean([FeretP.MaxFeretDiameterMicron]);
ST.StDev_MaxFeretDiameter_micron = std([FeretP.MaxFeretDiameterMicron]);
ST.MinFeretDiameter_micron = mean([FeretP.MinFeretDiameterMicron]);
ST.StDev_MinFeretDiameter_micron = std([FeretP.MinFeretDiameterMicron]);

ST = struct2table(ST);

```



storing info

```

cd NodesXY
mkdir L1
cd L1
save ST ST
save FeretP FeretP

```

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A.I.2 RotationSlices

```
% Rotationslices rotates X-CT images by a desired angle gamma. This creates a 3D
% matrix with rotated X-CT images, which are then cropped to save memory.

% Img_FolderPath = 'C:\Users\prb19227\Documents\MATLAB';
% Img_LatticeName = 'C2_RedHalf';

Img_FolderPath = 'C:\Users\beatr\OneDrive - University of
Strathclyde\PhD\Documents\MATLAB';
Img_LatticeName = 'L8_lattice'; %'L1_lattice'; 'L2_lattice' 'L3_lattice' 'L4_lattice'
'L6_lattice' 'L7_lattice'
Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);

imds = imageDatastore(Img_FullFilePath,...
    'IncludeSubfolders',true,'FileExtensions','.tiff','LabelSource','foldernames');
```

Properties of the stack of images

```
NbImg_Z = length(imds.Files);
alpha = 0; %roation angle determined using Dragonfly to have struts parallel to x-y
grid
beta = 45; %roation angle desired
gamma = alpha + beta; %total rotation angle
```

Scale management

```
% PixelWidth_micron = 10.954; %(1 pixel = 10.954 micron for X-CT
batch1)
```

```
PixelWidth_micron = 7.24575; %(1 pixel = 7.24 micron)
```

Rotation of images of angle gamma

```
% Initialization of R - array with rotated images
Img_1 = readimage(imds,27);
Img_R1 = imrotate(Img_1, gamma, 'bilinear');
[ImgHeight_pixel, Imgwidth_pixel, ~] = size(Img_R1);
R = false(ImgHeight_pixel, Imgwidth_pixel, NbImg_Z);
R(:, :, 1) = Img_R1;
ROT = R(:, :, 1);
warning('off','all')
Fig_Orig = figure('NumberTitle', 'off', 'name', 'Original', 'Color', [1 1 1]);%
Create figure
set(Fig_Orig, 'Units', 'normalized');
%     ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
%     set(Fig_Area, 'OuterPosition', ImgFigOutPosition); % Uncomment to define
figure position
Axes_Orig = axes(Fig_Orig); % Create axes
cla(Axes_Orig, 'reset'); % Reset axes (good for memory)
hold(Axes_Orig, 'on')
imshow(Img_1, 'Parent', Axes_Orig)
```

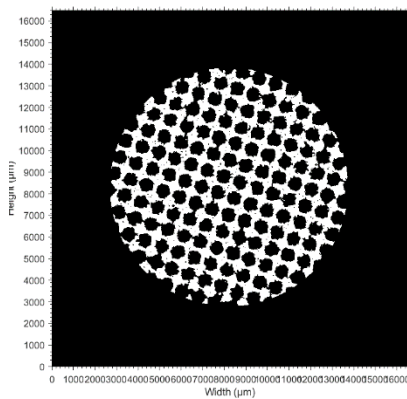
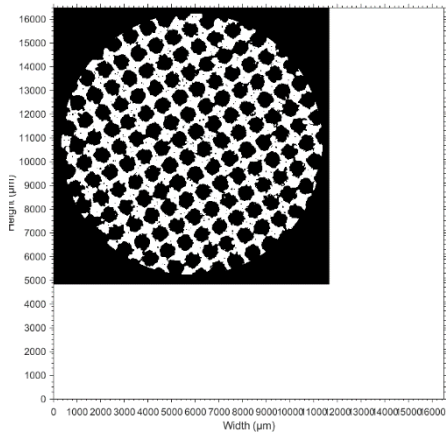
```

%----- Add scale in  $\mu\text{m}$  to x & y axis
micron_Ticks_Step = 1000;
Fctn_AddScale(Axes_Orig, micron_Ticks_Step, PixelWidth_micron, ImgHeight_pixel,
Imgwidth_pixel)

% Check on Rotation
Fig_Crop = figure('NumberTitle', 'off', 'name', 'Rotated', 'Color', [1 1 1]);%
Create figure
set(Fig_Crop, 'Units', 'normalized');
%   ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
%   set(Fig_Area, 'OuterPosition', ImgFigOutPosition); % Uncomment to define
figure position
Axes_Crop = axes(Fig_Crop); % Create axes
cla(Axes_Crop, 'reset'); % Reset axes (good for memory)
hold(Axes_Crop, 'on')
imshow(ROT, 'Parent', Axes_Crop)
%----- Add scale in  $\mu\text{m}$  to x & y axis
micron_Ticks_Step = 1000;
Fctn_AddScale(Axes_Crop, micron_Ticks_Step, PixelWidth_micron, ImgHeight_pixel,
Imgwidth_pixel)

% return

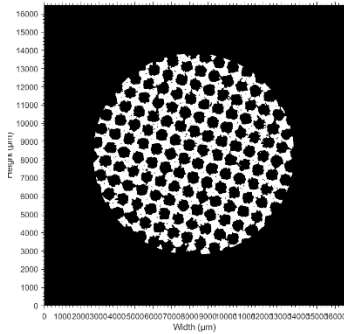
```



Run if happy

Rotating and storing rotated images in R

```
for img_i = 2:NbImg_Z
    Img = readimage(imds, img_i);
    Img_Ri = imrotate(Img, gamma, 'bilinear');
    R(:, :, img_i) = Img_Ri;
end
```



Cropping R to save memory

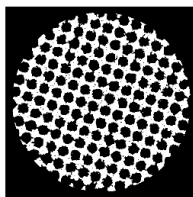
```
se_size = 200; % might need to be adjusted
Img_fill = imfill(imdilate(Img_R1, strel('disk', se_size)), 'holes');

%--- Retrieve region (= circle) properties
Img_fill_properties = regionprops('table', Img_fill, 'Area', 'Centroid');
Edge_Area = max(Img_fill_properties.Area);
Edge_Centre = Img_fill_properties.Centroid(Img_fill_properties.Area == Edge_Area, :);
Edge_Radius = round(sqrt(Edge_Area / pi())) - 1 * se_size; % Maybe need to change '1
* se_size' by '1.1 * se_size' or more

% Indexes to crop R
Safety_Factor = 50;
StartY = Edge_Centre(1) - Edge_Radius - Safety_Factor;
FinishY = Edge_Centre(1) + Edge_Radius + Safety_Factor;
SX = Edge_Centre(2) - Edge_Radius - Safety_Factor;
FX = Edge_Centre(2) + Edge_Radius + Safety_Factor;

%R_Crop
R_Crop = R(StartY:FinishY, SX:FX, :);

Fig_Orig = figure('NumberTitle', 'off', 'name', 'Rotated and Cropped', 'Color', [1 1
1]); % Create figure
imshow(R_Crop(:, :, 1))
```



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A.I.3 ExtractionSlicesXprimeZprime_DiagonalStruts

% ExtractionSlicesXprimeZprime_DiagonalStruts allows to extract the X-CT slices at 45
% degrees from the original XY plane. As such, it generates the X-CT slices that are
% orthogonal to the diagonal struts of the octet lattice. The output of this script
% represents the input for the AnalysisDiagonalFeatures_STRUTS_XYprimeslices_v1.

Housekeeping

```
clc  
warning('off','all')
```

INPUT NEEDED

loading of the 3D array containing the images of interest

```
%load('R_rotated_images_L1.mat','R')  
load R_Crop_C2; % R_Crop_L1 R_Crop_L2 R_Crop_L3 R_Crop_L4 R_Crop_L5 R_Crop_L8  
% R = R_Crop; % R-Crop is a variable stored in R_Crop_LX  
% Choose a name for the slices  
Name_Img = 'C2_Slices_45XYprime_'; % TO BE CHANGES ACCORDING TO Img_LatticeName  
'L2_Slices_45XYprime_' 'L3_Slices_45XYprime_'
```

**decide number of lattice cells to be analyzed in the thickness LUC
= Lattice Unit Cell**

```
LUC = 2;  
% UCS = Unit Cell Size in micron  
UCS = 1100; % 1 Unit Cell Size is 1,1 mm  
% X-CT Resolution  
% XCT_Res = 7.24575; %(1 pixel = 7.24 micron)  
XCT_Res = 10.954; %(1 pixel = 10.954 micron for X-CT batch1)
```

**Folder path for logical arrays containing info for images "Xprime-
Zprime" planes**

```
Img_FolderPath = 'C:\Users\beatr\OneDrive - University of  
Strathclyde\PhD\Documents\MATLAB';  
mkdir C2_45XYprimeDiagonalStruts  
% Img_LatticeName = 'L2_45XprimeZprimeDiagonalStruts';  
% Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);
```

size of images "Xprime-Zprime" planes

```
width = (LUC * (UCS/XCT_Res)); % Number of slices needed  
Hwidth = ceil(width/2); %Halfwidth - integer  
[~, R_width, ~] = size(R_Crop);  
CentreR = round(R_width/2);  
StartR = CentreR - Hwidth;  
FinishR = CentreR + Hwidth;
```

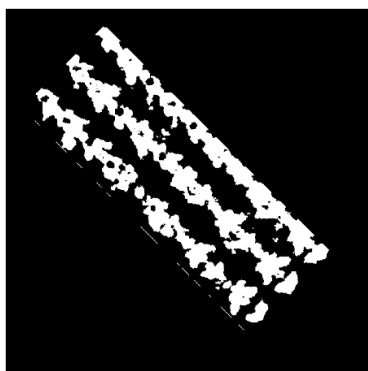
```

% check on rotation angle
i_ycheck = CentreR + 30;
img_ixz_ORGN = R_Crop(:,i_ycheck,:); %3D
img_ixz_ORGN = reshape(img_ixz_ORGN, size(img_ixz_ORGN,1), size(img_ixz_ORGN,3)); %2D
image
Fig_ORGN = figure('NumberTitle', 'off', 'name', 'Original', 'Color', [1 1 1]);%
Create figure
imshow(img_ixz_ORGN)

delta = 45; %to be changed

img_ixz_ROT = imrotate(img_ixz_ORGN, delta, 'bilinear');
Fig_RotDelta = figure('NumberTitle', 'off', 'name', 'Rotated', 'Color', [1 1 1]);%
Create figure
imshow(img_ixz_ROT)

```



Preparation to store the images/array cut according to the new X-Z plane

(Xprime, Zprime)

```
YprimeSize = FinishR - StartR; % dimension of the array along Y prime
XprimeSize = size(img_ixz_ROT,1);
ZprimeSize = size(img_ixz_ROT,2);

D = false(XprimeSize, YprimeSize, ZprimeSize); % to extract slices

RR = false(XprimeSize, YprimeSize, ZprimeSize); % to create 3D matrix
% Let's go:
for i_y = StartR:FinishR
    img_ixz_old = R_Crop(:,i_y,:); %3D image
    img_ixz = reshape(img_ixz_old, size(img_ixz_old,1), size(img_ixz_old,3)); %2D
    image
    img_ixz_ROT = imrotate(img_ixz, delta, 'bilinear');
    img_ixz_ROT3D = reshape(img_ixz_ROT, size(img_ixz_ROT,1),1, size(img_ixz_ROT,2));
    RR(:,i_y,:) = reshape(img_ixz_ROT, size(img_ixz_ROT,1),1, size(img_ixz_ROT,2));
%creation of 3D matrix in the right orientation to then extract slices
end

clear R_Crop % to save memory
% Cropping RR for memory saving
% From half width in
% Zprime --> 25% in Zprime + direction and --> 25% in Zprime - direction;
% also in xprime direction: from half width in xprime --> 35% in xprime +
% direction and 35% xprime - direction.

% Size original RR
XprimeL = size(RR,1);
YprimeL = size(RR,2);
ZprimeL = size(RR,3);
```

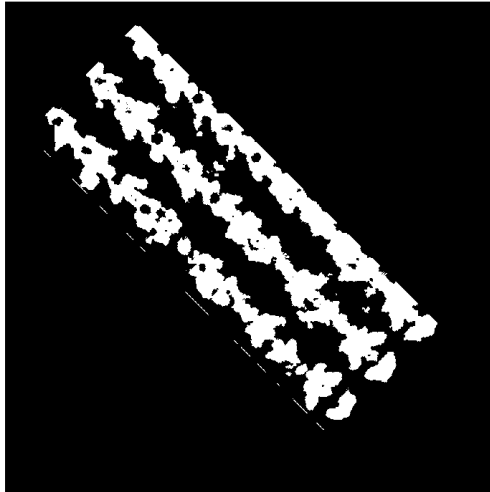
Centre Points and Range; Start and Finish slices

```
ZCentreSlice = ceil(ZprimeL/2);
Z25percent = ceil(ZprimeL/4);
Zstart = ZCentreSlice - Z25percent;
Zfinish = ZCentreSlice + Z25percent;

XCentreSlice = ceil(XprimeL/2);
X35percent = ceil(XprimeL*35/100);
Xstart = XCentreSlice - X35percent;
Xfinish = XCentreSlice + X35percent;

Ytempt1 = ceil(YprimeL - YprimeL*5/7); %need to be changed based on the dataset

RRCrop = RR(Xstart:Xfinish,Ytempt1:YprimeL, Zstart:Zfinish);
```



Clear RR to save memory

```
clear RR
```

Extraction slices from RRCrop

```
cd C2_45XyprimeDiagonalStruts % L2_45XyprimeDiagonalStruts

% We want slices that are perpendicular to xprime-Zprime Plane now - and
% parallel to xprime-Yprime
% So we want to go along Zprime to extract the images
for i_z = 1:size(RRCrop,3)
    img_axy = RRCrop(:,:, i_z); %2D image ???
    imshow(img_axy)
    number_img = string(i_z);
    Full_name = string(Name_Img + number_img);
    Full_name = char(Full_name);
    save(Full_name, 'img_axy');
end
```



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A.I.4 AnalysisDiagonalFeatures_STRUTS_XYprimeslices_v1

AnalysisDiagonalFeatures_STRUTS_XYprimeslices_v1 performs the automatic analysis and measurement of the diagonal struts of the lattices fabricated by L-PBF.

Housekeeping

```
%clf
clc
%clear all
warning('off','all')
```

Scale management

```
%Pixelwidth_micron = 7.24575; %(1 pixel = 7.24 micron)
Pixelwidth_micron = 10.954; %(1 pixel = 10.954 micron for X-CT batch1)
```

Choose what should be computed (= 1 only)

```
Compute_BlobAtEdgeFiltering = 0; % Remove blobs in touch with the edge (circular
shape only !!!)
Compute_BlobSizeColour = 1; % Colour blobs according to their area
Compute_BlobSizeFiltering = 1; % Filter blobs in touch with the outer circle
LightVersion = 1; %1 means NO figures are created; 0 means that Figures are generated
```

Creation of Data store to import all the segmented slices from X-CT for a single sample

```
% Folder path and Lattice Name (Name of the Folder containing images for
% single sample
Img_FolderPath = 'C:\Users\beatr\OneDrive - University of
Strathclyde\PhD\Documents\MATLAB';
Img_LatticeName = 'C2_45XYprimeDiagonalStruts'; % 'L1_45XprimeYprimeDiagonalStruts'
%'L1_45XprimeYprimeDiagonalStruts' 'L2_45XYprimeDiagonalStruts';
'L3_45XYprimeDiagonalStruts' 'L4_45XYprimeDiagonalStruts'
'L5_45XYprimeDiagonalStruts' 'L6_45XYprimeDiagonalStruts'
Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);

if ~isfolder(Img_FullFilePath) % the tilde '~' is a logical negation. So the if
condition is true (the file exist), the algorithm goes to ELSE condition.
    disp('Please updated the folder path and or folder name!')
    return
else
    imds = fileDatastore(Img_FullFilePath, 'ReadFcn', @load,
'FileExtensions',{'mat'});

    % Initialize a cell array to hold the data and counter |i|.
    dataarray = cell(numel(imds.Files), 1);
    i = 1;
    % Reset the datastore to the first file and read the files one at a time
    % until there is no data left. Assign the data to the array |dataarray|.
    reset(imds);
```



```

while hasdata(imds)
    dataarray{i} = read(imds);
    i = i+1;
end
end

```

IMAGE ANALYSIS START

Assumption image contains some objects (ones)

```
ImageContains1 = 1;
```

```
for i_img = 1:ceil(length(dataarray)/7)
```

```

    I = dataarray{i_img,1}; % reads the i-th matrix-image in the image store imds
    Img(:, :) = I.img_ixy(:, :);
    Img_bw = Img;
    number_img = string(i_img); %number of slice to put on the title of figures
    %Img_bw = Img == 255;% Convert image to binary

```

Remove blobs at edge

```

if Compute_BlobAtEdgeFiltering == 1
    se_size = 25;
    DispResult = 1; % Set 'DispResult = 1' if intermediate steps should be
displayed
    %[Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img_bw, se_size,
DispResult);
    [Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img, se_size, DispResult);
    Img2Use = Img_NoEdge;
else
    Img2Use = Img;
    %imshow(Img2Use)
end

```

Image properties

```

[ImgHeight_pixel, Imgwidth_pixel, ~] = size(Img2Use); % Size of the image Img_bw
Img_Labeled = bwlabel(Img2Use, 8); % 8 : connectivity %bwlabel LABELS connected
components in a binary image

```

Image analysis with region props

```

BLACK = false(ImgHeight_pixel, Imgwidth_pixel);

if Img2Use == BLACK
    %A(i_img,1) = 0;
    ImageContains1 = 0;
else
    %A(i_img,1) = 1;
    ImageContains1 = 1;
    Properties = regionprops('table', Img_Labeled , {'Area',

```

```

'PixelList','ConvexHull','MinorAxisLength','MajorAxisLength','Eccentricity'})); %
Changing 'all' by the selection of desired properties decrease computation time
    Properties.AreaMicron = Properties.Area .* ((PixelWidth_micron)^2); % creates
a coloumn in the TABLE Properties called "AreaMicron"
    SliceNumber_imgI = zeros(height(Properties),1);
    SliceNumber_imgI(:) = i_img; %it's a vector
    Properties.SliceNumber = SliceNumber_imgI;
end

```

Display image where the blobs are coloured according to their area

=> Permits to adjust filtering values

```

if Compute_BlobSizeColour == 1 && ImageContains1 ==1
    %--- 1st step : Convert data (µm²) to create coloured image (+ Conversion
pixel => µm)
    [ColourBlob_Img, BinLimits, cmap] = Fctn_ColourBlob_Area(Img2Use,
Properties.AreaMicron);
    % ColourBlob_Img : image
    % BinLimits : max and min area values
    % ColorMap : Matlab object permitting to manage image colours

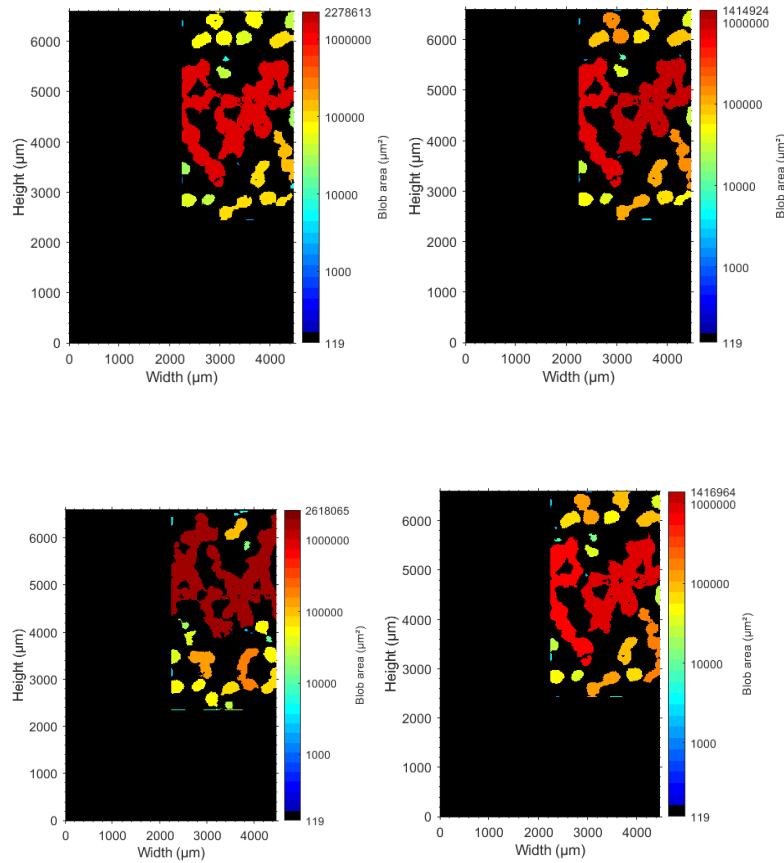
    %--- 2nd step : Create figure and axes, display image & define required
settings
    Fig_Strut = figure('NumberTitle', 'off', 'name', 'Blob area display of slice
' + number_img , 'color', [1 1 1]);% Create figure
    set(Fig_Strut, 'Units', 'normalized');
    %    ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
    %    set(Fig_Strut, 'OuterPosition', ImgFigOutPosition); % Uncomment to
define figure position
    Axes_SOI = axes(Fig_Strut); % Create axes
    cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
    hold(Axes_SOI, 'on')

    imshow(ColourBlob_Img, 'Parent', Axes_SOI)
    set(Axes_SOI, 'ColorScale', 'log', 'CLim', [BinLimits(1), BinLimits(2)],
'Colormap', cmap) % 'lin' <=> 'log'
    cBar = colorbar(Axes_SOI, 'AxisLocation', 'out'); % Display color bar
    set(cBar, 'Ticks', sort(unique([cBar.Limits, cBar.Ticks]))) % Display color
bar

    cBar.Label.String = 'Blob area (µm²)';
    cBar.Ruler.Scale = 'log'; % 'lin' <=> 'log'
    cBar.Ruler.MinorTick = 'on';

    %----- Add scale in µm to x & y axis
    micron_Ticks_Step = 1000;
    Fctn_AddScale(Axes_SOI, micron_Ticks_Step, PixelWidth_micron,
ImgHeight_pixel, Imgwidth_pixel)
end

```



Segmentation - Filtering : keep only the struts of interest

```

if Compute_BlobSizeFiltering == 1 && ImageContains1 == 1
    %--- Trigger definition
    trig_low_Micron = 50500;
    trig_high_Micron = 80000;

    %--- Filtering : Identify the struts of interest (SOIs)
    Index_SOI = find((Properties.AreaMicron >= trig_low_Micron) &
        (Properties.AreaMicron <= trig_high_Micron)); % SOI = Strut Of Interest
    Mask_SOI = ismember(Img_Labeled, Index_SOI); % Extract only those blobs that
    meet our criteria
    %     figure, imshow(Mask_SOI);

    %--- Extract boundaries of the SOI
    Boundaries_SOI = bwboundaries(Mask_SOI, 8, 'noholes'); % 8 : connectivity

    if LightVersion == 0

        %--- Display result
        %----- Figure & axes initialisation
        Fig_SOI = figure('NumberTitle', 'off', 'name', 'SOI display ' + number_img,
            'color', [1 1 1]); % Create figure
        set(Fig_SOI, 'Units', 'normalized');
        %     ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
    
```

```

position
    %      set(Fig_Area, 'OuterPosition', ImgFigOutPosition); % Uncomment to
define figure position
    Axes_SOI = axes(Fig_SOI); % Create axes
    cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
    hold(Axes_SOI, 'on')

    %----- Display background image (starting image)
    imshow(Img, 'Parent', Axes_SOI)

    %----- Add boundaries
    BoundaryColour = 'r'; % (r = red, g = green, ...)
    BoundaryThickness = 2;
    for i_Boundary = 1 : size(Boundaries_SOI, 1)
        Boundary_i = Boundaries_SOI{i_Boundary};
        plot(Axes_SOI, Boundary_i(:,2), Boundary_i(:,1), BoundaryColour,
'Linewidth', BoundaryThickness);
    end
    hold(Axes_SOI, 'off')

    %----- Add scale in  $\mu\text{m}$  to x & y axis
    micron_Ticks_Step = 1000;
    Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron,
ImgHeight_pixel, Imgwidth_pixel)
end

end

```

Analysis of the regions with Ellipse Axes, Feret Diameters and Bounding box

The function FeretPropertiesBEA5 exploited and modified the FeretProperties function available at <https://uk.mathworks.com/help/matlab/ref/waitbar.html>

```

if ImageContains1 == 1
    Props_SOI = Properties(Index_SOI, :); % Creates table with regionProps only for
the indexed SOIs
    % Props_SOI_ConvexHullmicron = [Props_SOI.ConvexHull{:,1}].*Pixelwidth_micron;

    % MinorAxisLength (or MajorAxisLength) is the Length (in pixels) of the
    % minor axis (or major axis) of the ellipse that has the same normalized
    % second central moments as the region, returned as a scalar. Second
    % Central Moments are IMAGE PROPERTIES often used after segmentation to
    % help the analysis.
    Props_SOI.MajorAxisLengthMicron = Props_SOI.MajorAxisLength .*
(Pixelwidth_micron); %adding Minor and Major Axis of the Ellipse in micron
    Props_SOI.MinorAxisLengthMicron = Props_SOI.MinorAxisLength .*
(Pixelwidth_micron);

    P_SOI = table2struct(Props_SOI); % creates a STRUCTURE

    %--- Store the filtered struts in singular vectors and cells. To do this, it's
better
    % to store the data in separated vector and then to recombine the

```

```

% table. We need to do this so we have a full table that contains ALL
% the struts (from all the slices), rather than having many P_SOI
% struts that are relative only to one single slice.

SliceNumberV = Props_SOI.SliceNumber(:);
AreaMicronV = Props_SOI.AreaMicron(:);
ConvexHullC = Props_SOI.ConvexHull(:);
PixelListC = Props_SOI.PixelList(:);
MajourAxisLengthMicronC = Props_SOI.MajourAxisLengthMicron(:);
MinorAxisLengthMicronC = Props_SOI.MinorAxisLengthMicron(:);
EccentricityC = Props_SOI.Eccentricity(:);

if i_img == 1
    i0 = 0;
    i = i0 +1;
    j = i + length(SliceNumberV) -1;

    SliceNumber(i:1:j,1) = SliceNumberV(:);
    AreaMicron(i:1:j,1) = AreaMicronV(:);
    ConvexHull(i:1:j,1) = ConvexHullC(:);
    PixelList(i:1:j,1) = PixelListC(:);
    Eccentricity(i:1:j,1) = EccentricityC(:);
    MajourAxisLengthMicron(i:1:j,1) = MajourAxisLengthMicronC(:);
    MinorAxisLengthMicron(i:1:j,1) = MinorAxisLengthMicronC(:);
    i0 = j;
else
    i0 = j;
    i = i0 +1;
    j = i + length(SliceNumberV) -1;
    SliceNumber(i:1:j,1) = SliceNumberV(:);
    AreaMicron(i:1:j,1) = AreaMicronV(:);
    ConvexHull(i:1:j,1) = ConvexHullC(:);
    PixelList(i:1:j,1) = PixelListC(:);
    Eccentricity(i:1:j,1) = EccentricityC(:);
    MajourAxisLengthMicron(i:1:j,1) = MajourAxisLengthMicronC(:);
    MinorAxisLengthMicron(i:1:j,1) = MinorAxisLengthMicronC(:);
    i0 = j;
end

%--- Making the table with all the struts of interest
T = table(SliceNumber, AreaMicron,ConvexHull,PixelList, Eccentricity,
MajourAxisLengthMicron, MinorAxisLengthMicron);
T = table2struct(T);

%--- Calculating Feret Properties
FeretP = feretPropertiesBEA5(T, Pixelwidth_micron);
% --- stuff
endpointsMAXferet = {FeretP(:).MaxFeretDiameterEndpoints}; % in pixel
endpointsMAXferetX = cell(size(endpointsMAXferet));
for xx=1:size(endpointsMAXferet,2)
    endpointsMAXferetX{1,xx} = endpointsMAXferet{1,xx}{1,1}(:,1);
end

endpointsMAXferetY = cell((endpointsMAXferet));

```

```

for xx=1:size(endpointsMAXferet,2)
    endpointsMAXferetY{1,xx} = endpointsMAXferet{1,xx}{1,1}(:,2);
end

%--- Analysis of the region: estimation of minor axis of ellipses of each blob
from the area of the blob and the Max Feret diameter

%MinAxisEllipseFeret
A = zeros(size(FeretP,1),1);
a = zeros(size(FeretP,1),1);
b = zeros(size(FeretP,1),1); % HALF the minimum axis

for i_blob = 1 : size(FeretP,1)
    Data_Region_i = FeretP(i_blob);
    A(i_blob, 1) = Data_Region_i.AreaMicron;
    a(i_blob, 1) = Data_Region_i.MaxFeretDiameterMicron ./ 2;
    b = A(i_blob, 1) ./ (a * pi());
end

MinAxisEllipseMaxFeretMicron = b .* 2;

% Add this as a coloumn in FeretP
MinAxisEllipseC = num2cell(MinAxisEllipseMaxFeretMicron);
[FeretP.MinAxisEllipseMaxFeretMicron] = MinAxisEllipseC{:};

%--- Analysis of the region: estimation of the Equivalent Diameter
D_eq = zeros(size(FeretP,1),1);
for i_blob = 1 : size(FeretP,1)
    Data_Region_i = FeretP(i_blob);
    A(i_blob, 1) = Data_Region_i.AreaMicron;
    D_eq(i_blob,1) = sqrt((A(i_blob, 1) .* 4) ./ pi());
end

% Add this as a coloumn in FeretP
D_eqC = num2cell(D_eq);
[FeretP.D_eq] = D_eqC{:};

end

```

```

end

```

SUMMARY TABLE with Average and Standard Deviation measurements

ST contains: 1. Equivalent Diameter; 2. Orthogonal Diameter; 3. MaxFeretDiameter; 4. MinFeretDiameter; 5. MajorAxisLength; 6. MinorAxisLength; 7. MinorAxisLengthFromMaxFeretMicron

```

ST.DiagonalStrutsRelativeToLattice = convertCharsToStrings(Img_LatticeName);
ST.Equivalent_Diameter_micron = mean([FeretP.D_eq]);
ST.StDev_Equivalent_Diameter_micron = std([FeretP.D_eq]);
ST.Orthogonal_Diameter_micron = mean([FeretP.OrthogonalDiameterMicron]);
ST.StDev_Orthogonal_Diameter_micron = std([FeretP.OrthogonalDiameterMicron]);

```

```

ST.MaxFeretDiameter_micron = mean([FeretP.MaxFeretDiameterMicron]);
ST.StDev_MaxFeretDiameter_micron = std([FeretP.MaxFeretDiameterMicron]);
ST.MinFeretDiameter_micron = mean([FeretP.MinFeretDiameterMicron]);
ST.StDev_MinFeretDiameter_micron = std([FeretP.MinFeretDiameterMicron]);
ST.Majour_Axis_Micron = mean([FeretP.MajourAxisLengthMicron]);
ST.StDev_Majour_Axis_Micron = std([FeretP.MajourAxisLengthMicron]);
ST.Minor_Axis_Micron = mean([FeretP.MinorAxisLengthMicron]);
ST.StDev_Minor_Axis_Micron = std([FeretP.MinorAxisLengthMicron]);
ST.MinorAxis_FromMaxFeret_Micron = mean([FeretP.MinAxisEllipseMaxFeretMicron]);
ST.StDev_MinorAxis_FromMaxFeret_Micron = std([FeretP.MinAxisEllipseMaxFeretMicron]);

ST = struct2table(ST); %Transforming struct to table

```

HISTOGRAMS of VARIABLES of INTEREST

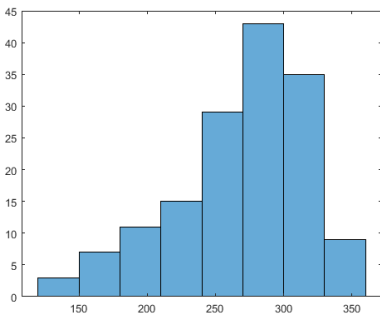
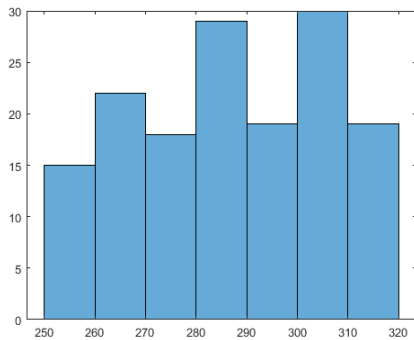
```

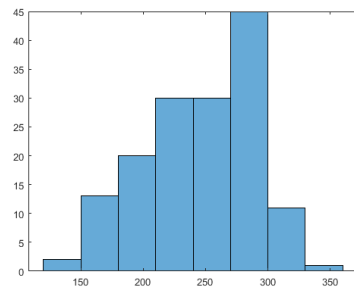
% Equivalent diameter
Fig_HistoEqD = figure('NumberTitle', 'off', 'name', 'Histogram Equivalent Diameter
Diagonal Struts (µm) ' + convertCharsToStrings(Img_LatticeName), 'color', [1 1 1]);
% Create figure
histogram([FeretP.D_eq]);

% Orthogonal diameter
HistoOrthoD = figure('NumberTitle', 'off', 'name', 'Histogram Orthogonal Diameter
Diagonal Struts (µm) ' + convertCharsToStrings(Img_LatticeName), 'color', [1 1 1]);
histogram([FeretP.OrthogonalDiameterMicron]);

% MinorAxisEllipse
HistoMinAxis = figure('NumberTitle', 'off', 'name', 'Histogram Minor Axis Ellipse for
Diagonal Struts (µm) ' + convertCharsToStrings(Img_LatticeName), 'color', [1 1 1]);
histogram([FeretP.MinorAxisLengthMicron]);

```





the analysis is saved in the folder "AnalysisDiagonalStrutsXYprime"
(From MATLAB folder)

```
cd AnalysisDiagonalStrutsXYprime
mkdir C2 % TO BE CHANGED
cd C2 % TO BE CHANGED
save ST ST
save FeretP FeretP
```

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A.I.5 ExtractionSlicesXZ

% ExtractionSlicesXZ allows to extract the X-CT slices at 90 degrees with respect to % the original XY plane of the X-CT data. As such, it generates the X-CT slices that % are orthogonal to the horizontal struts of the octet lattice. The output of this % script represents the input for the AnalysisHorizontalFeatures_Struts_XZ and % BC_Script_XZSlices_v9_from_NODES_XY.

Housekeeping

```
clc
warning('off','all')
```

INPUT NEEDED

loading of the 3D array containing the images of interest
load('R_rotated_images_L1.mat','R')

```
load('R_Crop_C2')
% R_Crop = R_Crop;
% Choose a name for the slices
Name_Img = 'C2_XZ_'; % TO BE CHANGES ACCORDING TO Img_LatticeName 'L1_Slices_45XZ_'
'L2_XZ_' 'L3_XZ_' 'L5_XZ_' 'L6_XZ_'
% decide number of lattice cells to be analysed in the thickness
% LUC = Lattice Unit Cell
LUC = 4;
% UCS = Unit Cell Size in micron
UCS = 1100; % 1 Unit Cell Size is 1,1 mm
% X-CT Resolution
% XCT_Res = 7.24575; %(1 pixel = 7.24 micron)
XCT_Res = 10.954; %(1 pixel = 10.954 micron for X-CT batch1)
```

Folder path for logical arrays containing info for images "x-z" planes

```
Img_FolderPath = 'C:\Users\prb19227\Documents\MATLAB';
Img_LatticeName = 'C2_RedHalf';
% Img_FolderPath = 'E:\OneDrive - University of Strathclyde\PhD\Documents\MATLAB'; %
'C:\Users\prb19227\Documents\MATLAB'
% Img_LatticeName = 'L8_45XZ'; % 'L1_45XZ' 'L2_45XZ'
Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);
```

size of images "x-z" planes

```
width = (LUC * (UCS/XCT_Res)); % Number of slices needed
Hwidth = ceil(width/2); %Halfwidth - integer
[~, R_width, ~] = size(R_Crop);
CentreR = round(R_width/2);
StartR = CentreR - Hwidth;
FinishR = CentreR + Hwidth;

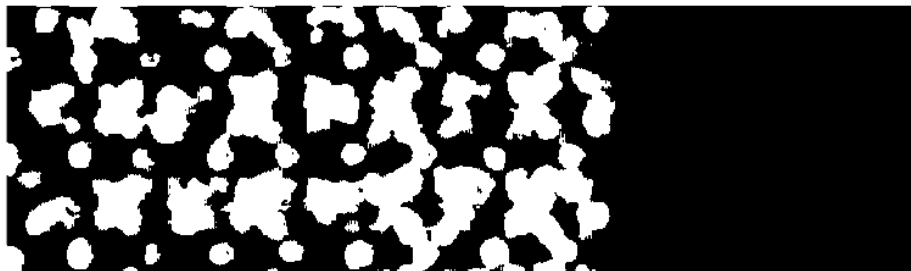
mkdir C2_XZ
```

```

cd 'C2_XZ'% L1_45XZ L2_XZ

for i_y = StartR:FinishR
    img_ixz_old = R_Crop(:,i_y,:); %3D image
    img_ixz = reshape(img_ixz_old, size(img_ixz_old,1), size(img_ixz_old,3)); %2D
    image
    img_ixz = img_ixz';
    imshow(img_ixz)
    number_img = string(i_y);
    Full_name = string(Name_Img + number_img);
    Full_name = char(Full_name);
    save(Full_name, 'img_ixz');
end

```



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A.I.6 BC_Script_XZSlices_v9_from_NODES_XY.m

```
% BC_Script_XZSlices_v9_from_NODES_XY performs the automatic geometrical  
% characterization of the lattice nodes that belong to the XZ plane.
```

Housekeeping

```
%clf  
clc  
%clear all  
warning('off','all')
```

Scale management

```
% Pixelwidth_micron = 7.24575; %(1 pixel = 7.24 micron)
```

```
Pixelwidth_micron = 10.954; %(1 pixel = 10.954 micron for X-CT batch1)
```

Choose what should be computed (= 1 only)

```
Compute_BlobAtEdgeFiltering = 0; % Remove blobs in touch with the edge (circular  
shape only !!!)  
Compute_BlobSizeColour = 0; % Colour blobs according to their area  
Compute_BlobSizeFiltering = 1; % Filter blobs in touch with the outer circle  
  
LightVersion = 1; %1 means NO figures are created; 0 means that Figures are generated
```

Creation of Data store to import all the segmented slices from X-CT for a single sample

```
% Folder path and Lattice Name (Name of the Folder containing images for  
% single sample  
  
Img_FolderPath = 'E:\OneDrive - University of Strathclyde\PhD\Documents\MATLAB';  
% 'C:\Users\prb19227\Documents\MATLAB'  
Img_LatticeName = 'C2_XZ'; % 'L1_Slices_XZ' 'L1-Lattice' 'L2_XZ' 'L3_XZ' 'L4_XZ'  
% Img_FolderPath = 'C:\Users\prb19227\Documents\MATLAB';  
% Img_LatticeName = 'L1_45XZ';  
Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);  
  
if ~isfolder(Img_FullFilePath) % the tilde '~' is a logical negation. So the if  
condition is true (the file exist), the algorithm goes to ELSE condition.  
    disp('Merci de mettre à jour le chemin d'accès au fichier bordel de merde !')  
    return  
else  
    imds = fileDatastore(Img_FullFilePath, 'ReadFcn', @load,  
        'FileExtensions',{'mat'});  
  
    % Initialize a cell array to hold the data and counter |i|.   
    dataarray = cell(numel(imds.Files), 1);  
    i = 1;  
    % Reset the datastore to the first file and read the files one at a time
```

```

% until there is no data left. Assign the data to the array |dataarray|.
reset(imds);
while hasdata(imds)
    dataarray{i} = read(imds);
    i = i+1;
end
end

```

Merci de mettre à jour le chemin d'accès au fichier bordel de merde !

IMAGE ANALYSIS START

Assumption image contains some objects (ones)

```

ImageContains1 = 1;

for i_img = 200:length(dataarray)

    I = dataarray{i_img,1}; % reads the i-th matrix-image in the image store imds
    Img(:, :) = I.img_ixz(:, :);
    Img_bw = Img;
    number_img = string(i_img); %number of slice to put on the title of figures
    %Img_bw = Img == 255;% Convert image to binary

```

Remove blobs at edge

```

if Compute_BlobAtEdgeFiltering == 1
    se_size = 25;
    DispResult = 1; % Set 'DispResult = 1' if intermediate steps should be
    displayed
    % [Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img_bw, se_size,
    DispResult);
    [Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img, se_size, DispResult);
    Img2Use = Img_NoEdge;
else
    Img2Use = Img;
    %imshow(Img2Use)
end

```

Image properties

```

[ImgHeight_pixel, Imgwidth_pixel, ~] = size(Img2Use); % Size of the image Img_bw
Img_Labeled = bwlabel(Img2Use, 8); % 8 : connectivity %bwlabel LABELS connected
components in a binary image

```

Image analysis with region props

```

BLACK = false(ImgHeight_pixel, Imgwidth_pixel);

if Img2Use == BLACK
    %A(i_img,1) = 0;
    ImageContains1 = 0;

```

```

else
    %A(i_img,1) = 1;
    ImageContains1 = 1;
    Properties = regionprops('table', Img_Labeled , {'Area',
'PixelList','ConvexHull','MinorAxisLength','MajorAxisLength','Eccentricity'}); %
Changing 'all' by the selection of desired properties decrease computation time
    Properties.AreaMicron = Properties.Area .* ((Pixelwidth_micron)^2); % creates
a coloumn in the TABLE Properties called "AreaMicron"
    SliceNumber_imgI = zeros(height(Properties),1);
    SliceNumber_imgI(:) = i_img; %it's a vector
    Properties.SliceNumber = SliceNumber_imgI;
end

```

Display image where the blobs are coloured according to their area

=> Permits to adjust filtering values

```

if Compute_BlobSizeColour == 1 && ImageContains1 ==1
    %--- 1st step : Convert data (µm²) to create coloured image (+ Conversion
pixel => µm)
    [ColourBlob_Img, BinLimits, cmap] = Fctn_ColourBlob_Area(Img2Use,
Properties.AreaMicron);
    % ColourBlob_Img : image
    % BinLimits : max and min area values
    % ColorMap : Matlab object permitting to manage image colours

    %--- 2nd step : Create figure and axes, display image & define required
settings

    Fig_Strut = figure('NumberTitle', 'off', 'name', 'Blob area display of slice
' + number_img, 'Color', [1 1 1]);% Create figure
    set(Fig_Strut, 'Units', 'normalized');
    %     ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
    %     set(Fig_Strut, 'OuterPosition', ImgFigOutPosition); % Uncomment to
define figure position
    Axes_SOI = axes(Fig_Strut); % Create axes
    cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
    hold(Axes_SOI, 'on')

    imshow(ColourBlob_Img, 'Parent', Axes_SOI)
    set(Axes_SOI, 'ColorScale', 'log', 'CLim', [BinLimits(1), BinLimits(2)],
'colormap', cmap) % 'lin' <=> 'log'
    cBar = colorbar(Axes_SOI, 'AxisLocation', 'out'); % Display color bar
    set(cBar, 'Ticks', sort(unique([cBar.Limits, cBar.Ticks]))) % Display color
bar

    cBar.Label.String = 'Blob area (µm²)';
    cBar.Ruler.Scale = 'log'; % 'lin' <=> 'log'
    cBar.Ruler.MinorTick = 'on';

    %----- Add scale in µm to x & y axis
    micron_Ticks_Step = 1000;
    Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron,

```

```

ImgHeight_pixel, Imgwidth_pixel)
end

```

Segmentation - Filtering : keep only the struts of interest

```

if Compute_BlobSizeFiltering == 1 && ImageContains1 ==1
    %--- Trigger definition
    trig_low_Micron = 150000;
    trig_high_Micron = 350000;

    %--- Filtering : Identify the struts of interest (SOIs)
    Index_SOI = find((Properties.AreaMicron >= trig_low_Micron) &
(Properties.AreaMicron <= trig_high_Micron)); % SOI = Strut Of Interest
    Mask_SOI = ismember(Img_Labeled, Index_SOI); % Extract only those blobs that
meet our criteria
    %     figure, imshow(Mask_SOI);

    %--- Extract boundaries of the SOI
    Boundaries_SOI = bwboundaries(Mask_SOI, 8, 'noholes'); % 8 : connectivity

    if LightVersion == 0

        %--- Display result
        %----- Figure & axes initialisation
        Fig_SOI = figure('NumberTitle', 'off', 'name', 'SOI display ' + number_img,
'color', [1 1 1]);% Create figure
        set(Fig_SOI, 'Units', 'normalized');
        %     ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
        %     set(Fig_Area, 'OuterPosition', ImgFigOutPosition); % Uncomment to
define figure position
        Axes_SOI = axes(Fig_SOI); % Create axes
        cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
        hold(Axes_SOI, 'on')

        %----- Display background image (starting image)
        imshow(Img, 'Parent', Axes_SOI)

        %     %----- Add boundaries
        %     BoundaryColour = 'r'; % (r = red, g = green, ...)
        %     BoundaryThickness = 2;
        %     for i_Boundary = 1 : size(Boundaries_SOI, 1)
        %         Boundary_i = Boundaries_SOI{i_Boundary};
        %         plot(Axes_SOI, Boundary_i(:,2), Boundary_i(:,1), BoundaryColour,
'Linewidth', BoundaryThickness);
        %     end
        %     hold(Axes_SOI, 'off')

        imgoverlay = imoverlay(Img_bw, Mask_SOI, 'yellow');
        imshow(imgoverlay, 'Parent', Axes_SOI);
        hold(Axes_SOI, 'on')

        %----- Add scale in µm to x & y axis
        micron_Ticks_Step = 1000;
        Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron,

```

```

    ImgHeight_pixel, Imgwidth_pixel)
        end

    end
end

```

Analysis of the regions with Ellipse Axes, Feret Diameters and Bounding box

<https://uk.mathworks.com/help/matlab/ref/waitbar.html> and modified

```

if ImageContains1 == 1
    Props_SOI = Properties(Index_SOI, :); % Creates table with regionProps only for
the indexed SOIs
    % Props_SOI_ConvexHullmicron = [Props_SOI.ConvexHull{:,1}].*Pixelwidth_micron;

    % MinorAxisLength (or MajorAxisLength) is the Length (in pixels) of the
    % minor axis (or major axis) of the ellipse that has the same normalized
    % second central moments as the region, returned as a scalar. Second
    % Central Moments are IMAGE PROPERTIES often used after segmentation to
    % help the analysis.
    Props_SOI.MajorAxisLengthMicron = Props_SOI.MajorAxisLength .*
(Pixelwidth_micron); %adding Minor and Major Axis of the Ellipse in micron
    Props_SOI.MinorAxisLengthMicron = Props_SOI.MinorAxisLength .*
(Pixelwidth_micron);

    P_SOI = table2struct(Props_SOI); % creates a STRUCTURE

    %--- Store the filtered struts in singular vectors and cells. To do this, it's
better
    %to store the data in separated vector and then to recombine the table

    SliceNumberV = Props_SOI.SliceNumber(:);
    AreaMicronV = Props_SOI.AreaMicron(:);
    ConvexHullC = Props_SOI.ConvexHull(:);
    PixelListC = Props_SOI.PixelList(:);

    if i_img == 1
        i0 = 0;
        i = i0 +1;
        j = i + length(SliceNumberV) -1;

        SliceNumber(i:1:j,1) = SliceNumberV(:);
        AreaMicron(i:1:j,1) = AreaMicronV(:);
        ConvexHull(i:1:j,1) = ConvexHullC(:);
        PixelList(i:1:j,1) = PixelListC(:);
        i0 = j;
    else
        i0 = j;
        i = i0 +1;
        j = i + length(SliceNumberV) -1;
        SliceNumber(i:1:j,1) = SliceNumberV(:);
        AreaMicron(i:1:j,1) = AreaMicronV(:);
        ConvexHull(i:1:j,1) = ConvexHullC(:);
        PixelList(i:1:j,1) = PixelListC(:);
    end
end

```

```

        i0 = j;
    end

    %--- Making the table with all the struts of interest
    T = table(SliceNumber, AreaMicron,ConvexHull,PixelList);
    T = table2struct(T);

    %--- TRIAL WITH NEW ORTHOGONAL DIAMETER WITH feretPropertiesBEA5
    % FeretP = feretPropertiesBEA4(P_SOI); BEA 4 works in script v5
    FeretP = feretPropertiesBEA5(T, Pixelwidth_micron);
    % --- stuff
    endpointsMAXferet = {FeretP(:).MaxFeretDiameterEndpoints}; % in pixel
    endpointsMAXferetX = cell(size(endpointsMAXferet));
    for xx=1:size(endpointsMAXferet,2)
        endpointsMAXferetX{1,xx} = endpointsMAXferet{1,xx}{1,1}(:,1);
    end

    endpointsMAXferetY = cell((endpointsMAXferet));
    for xx=1:size(endpointsMAXferet,2)
        endpointsMAXferetY{1,xx} = endpointsMAXferet{1,xx}{1,1}(:,2);
    end

    %--- Analysis of the region: estimation of minor axis of ellipses of each blob
    from the area of the blob and the Max Feret diameter

    %MinAxisEllipseFeret
    A = zeros(size(FeretP,1),1);
    a = zeros(size(FeretP,1),1);
    b = zeros(size(FeretP,1),1); % HALF the minimum axis

    for i_blob = 1 : size(FeretP,1)
        Data_Region_i = FeretP(i_blob);
        A(i_blob, 1) = Data_Region_i.AreaMicron;
        a(i_blob, 1) = Data_Region_i.MaxFeretDiameterMicron ./ 2;
        b = A(i_blob, 1) ./ (a * pi());
    end

    MinAxisEllipseMaxFeretMicron = b .* 2;

    % Add this as a coloumn in FeretP
    MinAxisEllipseC = num2cell(MinAxisEllipseMaxFeretMicron);
    [FeretP.MinAxisEllipseMaxFeretMicron] = MinAxisEllipseC{:};

    %--- Analysis of the region: estimation of the Equivalent Diameter
    D_eq = zeros(size(FeretP,1),1);
    for i_blob = 1 : size(FeretP,1)
        Data_Region_i = FeretP(i_blob);
        A(i_blob, 1) = Data_Region_i.AreaMicron;
        D_eq(i_blob,1) = sqrt((A(i_blob, 1) .* 4) ./ pi());
    end

    % Add this as a coloumn in FeretP
    D_eqC = num2cell(D_eq);
    [FeretP.D_eq] = D_eqC{:};

end

```



```
end
```

Histogram for the properties of the slices of interest and average properties (Equivalent Diameter, Max Feret, OrthoFeret, etc)

```
% Equivalent diameter
Fig_Histo = figure('NumberTitle', 'off', 'name', 'Histogram Equivalent Diameter Node
XZ( $\mu$ m)', 'Color', [1 1 1]); % Create figure
histogram([FeretP.D_eq]);

Eq_D_avg = mean([FeretP.D_eq]);

% Orthogonal diameter
Fig_HistoOF = figure('NumberTitle', 'off', 'name', 'Histogram Orthogonal Diameter
Node XZ ( $\mu$ m)', 'Color', [1 1 1]);
histogram([FeretP.OrthogonalDiameterMicron]);

Ortho_avg = mean([FeretP.OrthogonalDiameterMicron]);
```

Summary Table ST (as BC_Script_v9_NODE_XY.m)

```
ST.DiagonalStrutsRelativeToLattice = convertCharsToStrings(Img_LatticeName);
ST.Equivalent_Diameter_micron = Eq_D_avg;
ST.EquivDiamtMicron_SD = std([FeretP.D_eq]);
ST.OrthoDMicron = Ortho_avg;
ST.OrthoDM_SD = std([FeretP.OrthogonalDiameterMicron]);
ST.MaxFeretDiameter_micron = mean([FeretP.MaxFeretDiameterMicron]);
ST.StDev_MaxFeretDiameter_micron = std([FeretP.MaxFeretDiameterMicron]);
ST.MinFeretDiameter_micron = mean([FeretP.MinFeretDiameterMicron]);
ST.StDev_MinFeretDiameter_micron = std([FeretP.MinFeretDiameterMicron]);
ST = struct2table(ST);
```

Storing info

```
cd NodesXZ
mkdir C2
cd C2
save ST ST
save FeretP FeretP
```

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A.I.7 AnalysisHorizontalFeatures_Struts_XZ.m

```
% AnalysisHorizontalFeatures_Struts_XZ performs the automatic geometrical  
% characterization of the lattice struts that belong to the XZ plane.
```

Housekeeping

```
%clf  
clc  
%clear all  
warning('off','all')
```

Scale management

```
PixelWidth_micron = 7.24575; %(1 pixel = 7.24 micron)
```

```
PixelWidth_micron = 10.954; %(1 pixel = 10.954 micron for X-CT batch1)
```

Choose what should be computed (= 1 only)

```
Compute_BlobAtEdgeFiltering = 0; % Remove blobs in touch with the edge (circular  
shape only !!!)  
Compute_BlobSizeColour = 1; % Colour blobs according to their area  
Compute_BlobSizeFiltering = 1; % Filter blobs in touch with the outer circle  
LightVersion = 1; %1 means NO figures are created; 0 means that Figures are generated
```

Creation of Data store to import all the segmented slices from X-CT for a single sample

```
% Folder path and Lattice Name (Name of the Folder containing images for  
% single sample  
Img_FolderPath = 'E:\OneDrive - University of Strathclyde\PhD\Documents\MATLAB';  
Img_LatticeName = 'C2_XZ'; % 'L1_Slices_XZ' 'L1_45XprimeYprimeDiagonalStruts';  
L2_45XYprimeDiagonalStruts'; 'L2_XZ' 'L3_XZ' 'L4_XZ' 'L6_XZ' 'L8_XZ'  
Img_FullFilePath = fullfile(Img_FolderPath, Img_LatticeName);  
  
if ~isfolder(Img_FullFilePath) % the tilde '~' is a logical negation. So the if  
condition is true (the file exist), the algorithm goes to ELSE condition.  
    disp('Please updated the folder path and or folder name!')  
    return  
else  
    imds = fileDatastore(Img_FullFilePath, 'ReadFcn', @load,  
    'FileExtensions',{'mat'});  
  
    % Initialize a cell array to hold the data and counter |i|.   
    dataarray = cell(numel(imds.Files), 1);  
    i = 1;  
    % Reset the datastore to the first file and read the files one at a time  
    % until there is no data left. Assign the data to the array |dataarray|.   
    reset(imds);  
    while hasdata(imds)  
        dataarray{i} = read(imds);
```

```

        i = i+1;
    end
end

```

Please update the folder path and or folder name!

IMAGE ANALYSIS START

Assumption image contains some objects (ones)

```

ImageContains1 = 1;

for i_img = 1:ceil(length(dataarray)/7)

    I = dataarray{i_img,1}; % reads the i-th matrix-image in the image store imds
    Img(:, :) = I.img_ixz(:, :); % I.img_ixz(:, :) for Lattice L2-L8
    Img_bw = Img;
    number_img = string(i_img); %number of slice to put on the title of figures
    %Img_bw = Img == 255;% Convert image to binary

```

Remove blobs at edge

```

if Compute_BlobAtEdgeFiltering == 1
    se_size = 25;
    DispResult = 1; % Set 'DispResult = 1' if intermediate steps should be
    displayed
    %[Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img_bw, se_size,
    DispResult);
    [Img_NoEdge, Edge_Radius] = Fctn_RemoveEdgeBlobs(Img, se_size, DispResult);
    Img2Use = Img_NoEdge;
else
    Img2Use = Img;
    %imshow(Img2Use)
end

```

Image properties

```

[ImgHeight_pixel, Imgwidth_pixel, ~] = size(Img2Use); % Size of the image Img_bw
Img_Labeled = bwlabel(Img2Use, 8); % 8 : connectivity %bwlabel LABELS connected
components in a binary image

```

Image analysis with region props

```

BLACK = false(ImgHeight_pixel, Imgwidth_pixel);

if Img2Use == BLACK
    %A(i_img,1) = 0;
    ImageContains1 = 0;
else
    %A(i_img,1) = 1;
    ImageContains1 = 1;
    Properties = regionprops('table', Img_Labeled , {'Area',

```

```

'PixelList','ConvexHull','MinorAxisLength','MajorAxisLength','Eccentricity'})); %
Changing 'all' by the selection of desired properties decrease computation time
Properties.AreaMicron = Properties.Area .* ((Pixelwidth_micron)^2); % creates
a coloumn in the TABLE Properties called "AreaMicron"
SliceNumber_imgI = zeros(height(Properties),1);
SliceNumber_imgI(:) = i_img; %it's a vector
Properties.SliceNumber = SliceNumber_imgI;
end

```

Display image where the blobs are coloured according to their area

=> Permit to adjust filtering values

```

if Compute_BlobSizeColour == 1 && ImageContains1 ==1
    %--- 1st step : Convert data (µm²) to create coloured image (+ Conversion
    pixel => µm)
    [ColourBlob_Img, BinLimits, cmap] = Fctn_ColourBlob_Area(Img2Use,
    Properties.AreaMicron);
    % ColourBlob_Img : image
    % BinLimits : max and min area values
    % ColorMap : Matlab object permitting to manage image colours

    %--- 2nd step : Create figure and axes, display image & define required
    settings
    Fig_Strut = figure('NumberTitle', 'off', 'name', 'Blob area display of slice
    ' + number_img , 'Color', [1 1 1]);% Create figure
    set(Fig_Strut, 'Units', 'normalized');
    %     ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
    position
    %     set(Fig_Strut, 'OuterPosition', ImgFigOutPosition); % Uncomment to
    define figure position
    Axes_SOI = axes(Fig_Strut); % Create axes
    cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
    hold(Axes_SOI, 'on')

    imshow(ColourBlob_Img, 'Parent', Axes_SOI)
    set(Axes_SOI, 'ColorScale', 'log', 'CLim', [BinLimits(1), BinLimits(2)],
    'colormap', cmap) % 'lin' <=> 'log'
    cBar = colorbar(Axes_SOI, 'AxisLocation', 'out'); % Display color bar
    set(cBar, 'Ticks', sort(unique([cBar.Limits, cBar.Ticks]))) % Display color
    bar

    cBar.Label.String = 'Blob area (µm²)';
    cBar.Ruler.Scale = 'log'; % 'lin' <=> 'log'
    cBar.Ruler.MinorTick = 'on';

    %----- Add scale in µm to x & y axis
    micron_Ticks_Step = 1000;
    Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron,
    ImgHeight_pixel, Imgwidth_pixel)
end

```

Segmentation - Filtering : keep only the struts of interest

```
if Compute_BlobSizeFiltering == 1 && ImageContains1 ==1
    %--- Trigger definition
    trig_low_Micron = 50500;
    trig_high_Micron = 80000;

    %--- Filtering : Identify the struts of interest (SOIs)
    Index_SOI = find((Properties.AreaMicron >= trig_low_Micron) &
(Properties.AreaMicron <= trig_high_Micron)); % SOI = Strut Of Interest
    Mask_SOI = ismember(Img_Labeled, Index_SOI); % Extract only those blobs that
meet our criteria
    %     figure, imshow(Mask_SOI);

    %--- Extract boundaries of the SOI
    Boundaries_SOI = bwboundaries(Mask_SOI, 8, 'noholes'); % 8 : connectivity

    if LightVersion == 0

        %--- Display result
        %----- Figure & axes initialisation
        Fig_SOI = figure('NumberTitle', 'off', 'name', 'SOI display ' + number_img,
'color', [1 1 1]);% Create figure
        set(Fig_SOI, 'Units', 'normalized');
        %     ImgFigOutPosition = [0.375 0.05 0.6 0.9]; % Uncomment to define figure
position
        %     set(Fig_Area, 'OuterPosition', ImgFigOutPosition); % Uncomment to
define figure position
        Axes_SOI = axes(Fig_SOI); % Create axes
        cla(Axes_SOI, 'reset'); % Reset axes (good for memory)
        hold(Axes_SOI, 'on')

        %----- Display background image (starting image)
        imshow(Img, 'Parent', Axes_SOI)

        %----- Add boundaries
        BoundaryColour = 'r'; % (r = red, g = green, ...)
        BoundaryThickness = 2;
        for i_Boundary = 1 : size(Boundaries_SOI, 1)
            Boundary_i = Boundaries_SOI{i_Boundary};
            plot(Axes_SOI, Boundary_i(:,2), Boundary_i(:,1), BoundaryColour,
'Linewidth', BoundaryThickness);
        end
        hold(Axes_SOI, 'off')

        %----- Add scale in  $\mu\text{m}$  to x & y axis
        micron_Ticks_Step = 1000;
        Fctn_AddScale(Axes_SOI, micron_Ticks_Step, Pixelwidth_micron,
ImgHeight_pixel, Imgwidth_pixel)
    end

end
```

Analysis of the regions with Ellipse Axes, Feret Diameters and Bounding box

<https://uk.mathworks.com/help/matlab/ref/waitbar.html> and modified

```
if ImageContains1 == 1
    Props_SOI = Properties(Index_SOI, :); % Creates table with regionProps only for
    the indexed SOIs
    % Props_SOI_ConvexHullmicron = [Props_SOI.ConvexHull{:,1}].*PixelWidth_micron;

    % MinorAxisLength (or MajorAxisLength) is the Length (in pixels) of the
    % minor axis (or major axis) of the ellipse that has the same normalized
    % second central moments as the region, returned as a scalar. Second
    % Central Moments are IMAGE PROPERTIES often used after segmentation to
    % help the analysis.
    Props_SOI.MajorAxisLengthMicron = Props_SOI.MajorAxisLength .*
    (PixelWidth_micron); %adding Minor and Major Axis of the Ellipse in micron
    Props_SOI.MinorAxisLengthMicron = Props_SOI.MinorAxisLength .*
    (PixelWidth_micron);

    P_SOI = table2struct(Props_SOI); % creates a STRUCTURE

    %--- Store the filtered struts in singular vectors and cells. To do this, it's
    better
    % to store the data in separated vector and then to recombine the
    % table. We need to do this so we have a full table that contains ALL
    % the struts (from all the slices), rather than having many P_SOI
    % struts that are relative only to one single slice.

    SliceNumberV = Props_SOI.SliceNumber(:);
    AreaMicronV = Props_SOI.AreaMicron(:);
    ConvexHullC = Props_SOI.ConvexHull(:);
    PixelListC = Props_SOI.PixelList(:);
    MajorAxisLengthMicronC = Props_SOI.MajorAxisLengthMicron(:);
    MinorAxisLengthMicronC = Props_SOI.MinorAxisLengthMicron(:);
    EccentricityC = Props_SOI.Eccentricity(:);

    if i_img == 1
        i0 = 0;
        i = i0 +1;
        j = i + length(SliceNumberV) -1;

        SliceNumber(i:1:j,1) = SliceNumberV(:);
        AreaMicron(i:1:j,1) = AreaMicronV(:);
        ConvexHull(i:1:j,1) = ConvexHullC(:);
        PixelList(i:1:j,1) = PixelListC(:);
        Eccentricity(i:1:j,1) = EccentricityC(:);
        MajorAxisLengthMicron(i:1:j,1) = MajorAxisLengthMicronC(:);
        MinorAxisLengthMicron(i:1:j,1) = MinorAxisLengthMicronC(:);
        i0 = j;
    else
        i0 = j;
        i = i0 +1;
        j = i + length(SliceNumberV) -1;
        SliceNumber(i:1:j,1) = SliceNumberV(:);
        AreaMicron(i:1:j,1) = AreaMicronV(:);
```

```

ConvexHull(i:1:j,1) = ConvexHullC(:);
PixelList(i:1:j,1) = PixelListC(:);
Eccentricity(i:1:j,1) = EccentricityC(:);
MajourAxisLengthMicron(i:1:j,1) = MajourAxisLengthMicronC(:);
MinorAxisLengthMicron(i:1:j,1) = MinorAxisLengthMicronC(:);
i0 = j;
end

%--- Making the table with all the struts of interest
T = table(SliceNumber, AreaMicron,ConvexHull,PixelList, Eccentricity,
MajourAxisLengthMicron, MinorAxisLengthMicron);
T = table2struct(T);

%--- Calculating Feret Properties
FeretP = feretPropertiesBEA5(T, PixelWidth_micron);
% --- stuff
endpointsMAXferet = {FeretP(:).MaxFeretDiameterEndpoints}; % in pixel
endpointsMAXferetX = cell(size(endpointsMAXferet));
for xx=1:size(endpointsMAXferet,2)
    endpointsMAXferetX{1,xx} = endpointsMAXferet{1,xx}{1,1}(:,1);
end

endpointsMAXferetY = cell((endpointsMAXferet));
for xx=1:size(endpointsMAXferet,2)
    endpointsMAXferetY{1,xx} = endpointsMAXferet{1,xx}{1,1}(:,2);
end

%--- Analysis of the region: estimation of minor axis of ellipses of each blob
from the area of the blob and the Max Feret diameter

%MinAxisEllipseFeret
A = zeros(size(FeretP,1),1);
a = zeros(size(FeretP,1),1);
b = zeros(size(FeretP,1),1); % HALF the minimum axis

for i_blob = 1 : size(FeretP,1)
    Data_Region_i = FeretP(i_blob);
    A(i_blob, 1) = Data_Region_i.AreaMicron;
    a(i_blob, 1) = Data_Region_i.MaxFeretDiameterMicron ./ 2;
    b = A(i_blob, 1) ./ (a * pi());
end

MinAxisEllipseMaxFeretMicron = b .* 2;

% Add this as a coloumn in FeretP
MinAxisEllipsec = num2cell(MinAxisEllipseMaxFeretMicron);
[FeretP.MinAxisEllipseMaxFeretMicron] = MinAxisEllipsec{:};

%--- Analysis of the region: estimation of the Equivalent Diameter
D_eq = zeros(size(FeretP,1),1);
for i_blob = 1 : size(FeretP,1)
    Data_Region_i = FeretP(i_blob);
    A(i_blob, 1) = Data_Region_i.AreaMicron;
    D_eq(i_blob,1) = sqrt((A(i_blob, 1) .* 4) ./ pi());
end

```

```

% Add this as a coloumn in FeretP
D_eqC = num2cell(D_eq);
[FeretP.D_eq] = D_eqC{:};

end

end

```

SUMMARY TABLE with Average and Standard Deviation measurements

ST contains: 1. Equivalent Diameter; 2. Orthogonal Diameter; 3. MaxFeretDiameter; 4. MinFeretDiameter; 5. MajourAxisLength; 6. MinorAxisLength; 7. MinorAxisLengthFromMaxFeretMicron

```

ST.DiagonalStrutsRelativeToLattice = convertCharsToStrings(Img_LatticeName);
ST.Equivalent_Diameter_micron = mean([FeretP.D_eq]);
ST.StDev_Equivalent_Diameter_micron = std([FeretP.D_eq]);
ST.Orthogonal_Diameter_micron = mean([FeretP.OrthogonalDiameterMicron]);
ST.StDev_Orthogonal_Diameter_micron = std([FeretP.OrthogonalDiameterMicron]);
ST.MaxFeretDiameter_micron = mean([FeretP.MaxFeretDiameterMicron]);
ST.StDev_MaxFeretDiameter_micron = std([FeretP.MaxFeretDiameterMicron]);
ST.MinFeretDiameter_micron = mean([FeretP.MinFeretDiameterMicron]);
ST.StDev_MinFeretDiameter_micron = std([FeretP.MinFeretDiameterMicron]);
ST.Majour_Axis_Micron = mean([FeretP.MajourAxisLengthMicron]);
ST.StDev_Majour_Axis_Micron = std([FeretP.MajourAxisLengthMicron]);
ST.Minor_Axis_Micron = mean([FeretP.MinorAxisLengthMicron]);
ST.StDev_Minor_Axis_Micron = std([FeretP.MinorAxisLengthMicron]);
ST.MinorAxis_FromMaxFeret_Micron = mean([FeretP.MinAxisEllipseMaxFeretMicron]);
ST.StDev_MinorAxis_FromMaxFeret_Micron = std([FeretP.MinAxisEllipseMaxFeretMicron]);

ST = struct2table(ST); %Transforming struct to table

```

HISTOGRAMS of VARIABLES of INTEREST

```

% Equivalent diameter
Fig_HistoEqD = figure('NumberTitle', 'off', 'name', 'Histogram Equivalent Diameter
Diagonal Struts (µm) ' + convertCharsToStrings(Img_LatticeName), 'Color', [1 1 1]);
% Create figure
histogram([FeretP.D_eq]);

% Orthogonal diameter
HistoOrthoD = figure('NumberTitle', 'off', 'name', 'Histogram Orthogonal Diameter
Diagonal Struts (µm) ' + convertCharsToStrings(Img_LatticeName), 'Color', [1 1 1]);
histogram([FeretP.OrthogonalDiameterMicron]);

% MinorAxisEllipse
HistoMinAxis = figure('NumberTitle', 'off', 'name', 'Histogram Minor Axis Ellipse for
Diagonal Struts (µm) ' + convertCharsToStrings(Img_LatticeName), 'Color', [1 1 1]);
histogram([FeretP.MinorAxisLengthMicron]);

```

the analysis is saved in the folder "AnalysisDiagonalStrutsXYprime" (From MATLAB folder)

```

cd AnalysisHorizontalStruts_XZ % AnalysisDiagonalStrutsXYprime
mkdir C2 % TO BE CHANGED

```



```
cd C2 % TO BE CHANGED  
save ST ST  
save FeretP FeretP
```

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A.I.8 feretPropertiesBEA5

The function allows to calculate the Feret diameters and the orthogonal diameter of isolated struts and nodes ("regions").

```
function T = feretPropertiesBEA5(T, Pixelwidth_micron)

% feretPropertiesBEA5 calculates the maximum and minimum Feret Diameters,
% as well as the orientation of these and other stuff. INPUT: T is the
% structure coming from RegionProps of an image; Pixelwidth_micron is the
% width of the pixel of the image.
% OUTPUT: T is the same input structure, it contains all the regions of
% interest and gives as an output with the additional fields relative to
% the Feret Properties.

% feret Properties initialization
maxfd = zeros(length(T),1);
maxfd_endpoints = cell(length(T),1);
maxfd_orientation = zeros(length(T),1);

minfd = zeros(length(T),1);
minfd_triangle_points = cell(length(T),1);
minfd_orientation = zeros(length(T),1);

minod = zeros(length(T),1);
minod_lower_points = cell(length(T),1);
minod_upper_points = cell(length(T),1);

minbb = cell(length(T),1);
minbb_a = zeros(length(T),1);

for k = 1:length(T) % k index (for each region found from regionProps)

    Data_Region_k = T(k);
    % this algorithm works on the Theorem 4,18, Preparata and Shamos,
    % Computational Geometry, 1985. It assumes the input image is convex.
    % Therefore, we need to work on ConvexHull of the strut.
    ConvexHull_k = Data_Region_k.ConvexHull;
    % figure, imshow(ConvexHull_k)

    polygon=polyshape(ConvexHull_k); % N.B. polyshape needs x and y coordinates
    V = polygon.Vertices; %this contains the COORDINATES of the vertices of the
    polygon
    pairs = antipodalPairsB(V); %antipodalPairs finds antipodal vertices (vertices
    are the input here, in coordinates)

    % MAX Feret Diameter
    [maxfd(k),maxfd_endpoints{k}] = maxFeretDiameter(V,pairs);
    points = maxfd_endpoints{k};
    e = points(2,:) - points(1,:);
    maxfd_orientation(k) = atan2d(e(2),e(1));

    % min Feret Diameter
```

```

% min Feret Diameter is
[minfd(k),minfd_triangle_points{k}] = minFeretDiameter(V,pairs);
points = minfd_triangle_points{k};
e = points(2,:) - points(1,:);
thetad = atan2d(e(2),e(1));
minfd_orientation(k) = mod(thetad + 180 + 90,360) - 180;

% orthogonal diameter
% takes in the vertices V and the orientation of the maximum Feret
% Diameter
[minod(k),minod_lower_points{k},minod_upper_points{k}] = ...
    feretDiameter(V, maxfd_orientation(k)+90);

% minimum area bounding box
[minbb{k},minbb_a(k)] = minAreaBoundingBox(V,pairs);

end

% Conversion of Max Feret, Min Feret, Ortho Feret from pixels to micron
maxfd = maxfd .* PixelWidth_micron;
minfd = minfd .* PixelWidth_micron;
minod = minod .* PixelWidth_micron;

% Conversion of vectors to cells (C) into cells with appropriate array
% dimension to be then stored in T, the structure containing the
% information of the regions of interest

maxfdC = num2cell(maxfd);
maxfd_endpoints = num2cell(maxfd_endpoints);
maxfd_orientation = num2cell(maxfd_orientation);
minfdC = num2cell(minfd);
minfd_triangle_points = num2cell(minfd_triangle_points);
minfd_orientation = num2cell(minfd_orientation);
minod = num2cell(minod);
minod_lower_points = num2cell(minod_lower_points);
minod_upper_points = num2cell(minod_upper_points);
minbb = num2cell(minbb);
minbb_a = num2cell(minbb_a);

% Creating additional fields to T structure (in pixel, some exceptions):

[T.MaxFeretDiameterMicron] = maxfdC{:}; %this is in micron
[T.MaxFeretDiameterEndpoints] = maxfd_endpoints{:};
[T.MaxFeretDiameterOrientation] = maxfd_orientation{:};
[T.MinFeretDiameterMicron] = minfdC{:}; %this is in micron
[T.MinFeretDiameterTrianglePoints] = minfd_triangle_points{:};
[T.MinFeretDiameterOrientation] = minfd_orientation{:};
[T.OrthogonalDiameterMicron] = minod{:}; %this is in micron
[T.OrthogonalDiameterLowerPoints] = minod_lower_points{:};
[T.OrthogonalDiameterUpperPoints] = minod_upper_points{:};
[T.MinAreaBoundingBox] = minbb{:};
[T.MinAreaBoundingBoxArea] = minbb_a{:};
end

```

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A.I.9 CompressionTestDataAnalysis

The script allows to import and analyse the data relative to the compression test of the L-PBF Zr702 bulk cylinder. The ultimate objectives of this script are: 1) plotting the Engineering Stress vs Engineering Strain curve, and 2) estimating the Elastic Modulus of the material.

Notes and comments:

1: TimeMachine and TimeDIC The loads (N) here imported were measured by the cell load in the compression testing machine; the sample length (mm) and sample length variations (mm) at specific times were derived from the Digital Image Correlation (DIC) analysis. Because these variables were measured by different tools, Loads vs time and lengths vs time are not expressed in synchron. In fact, there are two different "clocks": 1) for the testing machine (TimeMachine), 2) for the DIC (t_DIC). Moreover, the Loads are recorded at a higher frequency than the lengths, resulting into vectors of different sizes. As such, this script was designed to couple correctly Loads (from which Stresses are derived) and Lengths (from which Strains are derived), before performing any estimate of the elastic modulus.

2: Denoising of True Strain and Derivation of Engineering Strain True Strains were calculated, and outliers (noise) were removed and substituted with "filloutliers" function. The engineering strains were then calculated from the denoised True Strains.

DATA IMPORT -- Load data from machine (Load, ...)

```
CompTestData = xlsread('MT-0320Sp6withDICpluscyclic.xls',4); %Import from sheet 4
SampleDetails = xlsread('MT-0320Sp6withDICpluscyclic.xls',3); % Import from sheet 3
CorrectionCurve1 = xlsread('CorrectionCurveRTH13M2July2022no1.xls',2);
CorrectionCurve2 = xlsread('CorrectionCurveRTH13M2July2022no2.xls',2);
CorrectionCurve3 = xlsread('CorrectionCurveRTH13M2July2022no3.xls',2);

StdForce_N = CompTestData(:,2); % Loads (N)
StdTravel_mm = CompTestData(:,3); % Reading from Platens hence Inaccurate)
timeMachine = CompTestData(:,4); % Time (s) from the Testing Machine

% Load data from DIC (strain, time, ...)
[DIC,~] = importdata('Specimen_6_removingoutliers.txt');
DICData = DIC.data;
clear DIC
```

```

%DICData contains 1) Image n.; 2) time (s); 3) L(t) (mm); 4) dL(t) (mm);
%5) strain (s); 6) force (us); 7) stress(us/mm^2);

% extraction of data of interest form DIC
t_DIC = DICData(:,2); % Time (s) relative to the DIC analysis
L_t = DICData(:,3); % Instant Length (mm)
dL_t = DICData(:,4); % Instant Incremental Length (mm)

% Sample data extraction
% H0 (initial height, mm), D0 (initial diameter, mm), A0 (initial
% cross-section, mm^2), Test_last (s)
H0 = SampleDetails(1,1); % Initial height (mm)
A0 = SampleDetails(1,4); % initial cross-section (mm^2)

```

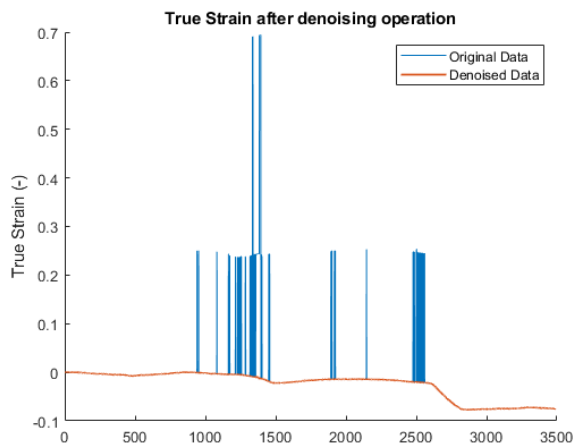
Calculation of True Strain from DIC Data and Engineering Strain (from Denoised True Strain)

```

TrueStrain = log(L_t./L_t(1,1)); % True Strain (-)
True_strain = filloutliers(TrueStrain,'nearest','mean'); % True Strain DENOISED by
removing and filling outliers with method 'nearest' and 'mean'
%strain = smoothdata(TrueStrain, 'lowess'); %lowess and movmean ----
%movmedian movmedian, gaussian --sgolay very bad
% strain = smoothdata(TrueStrain, 'movmean');
fig0 = figure('NumberTitle', 'off', 'name', 'True Strain after denoising operation',
'Color', [1 1 1]);
%plot(strain)
hold on
plot(TrueStrain)
hold on
plot(True_strain, 'Linewidth', 1.3)
title('True Strain after denoising operation')
ylabel('True Strain (-)')
legend({'Original Data', 'Denoised Data'}, 'Location', 'northeast')

Eng_Strain = exp(True_strain) -1; % Engineering strain from smoothened True Strain

```



Finding maxima and minima LOADS and the relative times in TIME MACHINE

```
fig1 = figure('NumberTitle', 'off', 'name', 'Load over MachineTime with Maximum and Minimum Points', 'Color', [1 1 1]);
plot(timeMachine, StdForce_N)
hold on
% title('Load over MachineTime with Maximum and Minimum Points')
xlabel('Time from Machine (s)')
ylabel('Load (N)')
hold on

Max = zeros(3,2);
Min = zeros(3,2);
M = zeros(3,2);
m = zeros(3,2);
TimeMaxMin = zeros(5,2); % save the time (s) in 1st coloumn and INDEX in time in 2nd coloumn (beginning and ending of the curve are ignored)
LoadMaxMin = zeros(5,2); % save the load (N) in 1st coloumn and INDEX in time in 2nd coloumn (beginning and ending of the curve are ignored)

% 1st Max
Low = 100;
High = 200;
IntMax1 = find(timeMachine >= Low & timeMachine <=High);
[Max(1,2), Max(1,1)] = max(StdForce_N(IntMax1));
tMax1 = Low + timeMachine(Max(1,1));
IndxT = find(StdForce_N == Max(1,2));
M(1,:) = [tMax1,Max(1,2)];
TimeMaxMin(1,:) = [tMax1, IndxT];
LoadMaxMin(1,:) = [Max(1,2),IndxT];
scatter(tMax1, Max(1,2), 'filled', 'MarkerFaceColor',[1 0.5 0])
hold on

% 2nd Max
Low = 200;
High = 400;
IntMax2 = find(timeMachine >= Low & timeMachine <=High);
[Max(2,2), Max(2,1)] = max(StdForce_N(IntMax2));
tMax2 = Low + timeMachine(Max(2,1));
M(2,:) = [tMax2,Max(2,2)];
IndxT = find(StdForce_N == Max(2,2));
TimeMaxMin(3,:) = [tMax2, IndxT];
LoadMaxMin(3,:) = [Max(2,2),IndxT];
scatter(tMax2,Max(2,2), 'filled', 'MarkerFaceColor',[1 0.5 0])
hold on

% 3rd Max
Low = 500;
High = 800;
IntMax3 = find(timeMachine >= Low & timeMachine <=High);
[Max(3,2), Max(3,1)] = max(StdForce_N(IntMax3));
TIME3Indx = find(StdForce_N == Max(3,2));
tMax3 = timeMachine(TIME3Indx);
M(3,:) = [tMax3,Max(3,2)];
IndxT = find(StdForce_N == Max(3,2));
TimeMaxMin(5,:) = [tMax3, IndxT];
```

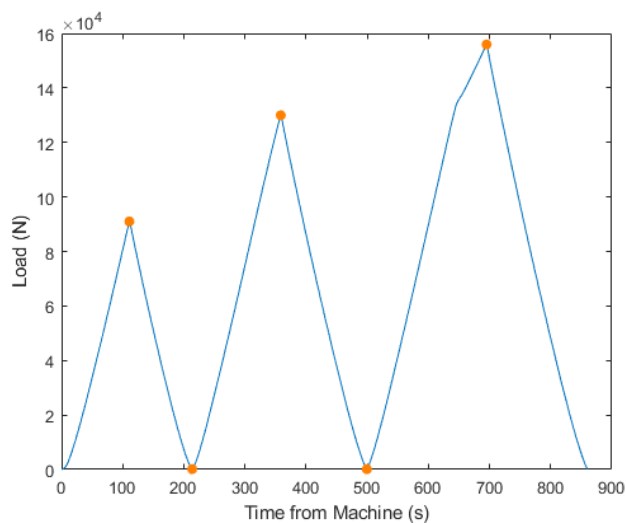
```

LoadMaxMin(5,:) = [Max(3,2),IndxT];
scatter(tMax3,Max(3,2), 'filled', 'MarkerFaceColor',[1 0.5 0])
hold on

% 1st Min
tLow = 200;
tHigh = 300;
IntMin1 = find(timeMachine >=tLow & timeMachine<= tHigh);
[Min(1,2), Min(1,1)] = min(StdForce_N(IntMin1));
IndxT = find(StdForce_N == Min(1,2));
tMax4 = timeMachine(IndxT);
TimeMaxMin(2,:) = [tMax4, IndxT];
tmin = tLow + timeMachine( Min(1,1));
t(3,1) = tmin;
TimeIndx = IntMin1(1)+ Min(1,1);
LoadMaxMin(2,:) = [Min(1,2),IndxT];
m(1,:) = [TimeIndx , Min(1,2) ];
scatter(tmin, Min(1,2), 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on

% 2nd Min
tLow = 400;
tHigh = 600;
IntMin1 = find(timeMachine >=tLow & timeMachine<= tHigh);
[Min(2,2), Min(2,1)] = min(StdForce_N(IntMin1));
IndxT = find(StdForce_N == Min(2,2));
tMax4 = timeMachine(IndxT);
TimeMaxMin(4,:) = [tMax4, IndxT];
tmin = tLow + timeMachine( Min(2,1));
t(4,1) = tmin;
TimeIndx = IntMin1(1)+ Min(2,1);
LoadMaxMin(4,:) = [Min(2,2),IndxT];
m(2,:) = [TimeIndx , Min(2,2) ];
scatter(tmin, Min(2,2), 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on

```



Find the maximum and minimum ENGINEERING STRAIN points and the relative time in DIC time

```
AbsoluteStrain = abs(Eng_Strain); % Absolute values of Engineering Strain (-)

timeMaxMinDIC = zeros(5,2); % save the time (s) in 1st coloumn and INDEX in time in
2nd coloumn (beginning and ending of the curve are ignored)
StrainMaxMin = zeros(5,2); % save the strain (-) in 1st coloumn and INDEX in time in
2nd coloumn (beginning and ending of the curve are ignored)

fig2 = figure('NumberTitle', 'off', 'name', 'True Strain over DICTime with Maximum
and Minimum Points', 'Color', [1 1 1]);
plot(t_DIC, AbsoluteStrain)
% title('Strain over DICTime with Maximum and Minimum Points')
xlabel('DIC Time (s)')
ylabel('Engineering Strain (-)')

hold on

% 1st Maximum Strain
tLow = 100;
tHigh = 200;
IntStr = find(t_DIC >=tLow & t_DIC <= tHigh);
[Max1, Indx1] = max(AbsoluteStrain(IntStr));
tMax1 = tLow + t_DIC(Indx1); %time (s)
TimeIndx = IntStr(1)+Indx1; %time indx (integer)
timeMaxMinDIC(1,:) = [tMax1, TimeIndx];
StrainMaxMin(1,:) = [Max1, TimeIndx];
scatter(tMax1, Max1, 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on

% 2nd Maximum Strain
tLow = 350;
tHigh = 370;
IntStr = find(t_DIC >=tLow & t_DIC <= tHigh);
[Max1, Indx1] = max(AbsoluteStrain(IntStr));
tMax1 = tLow + t_DIC(Indx1); %time (s)
TimeIndx = IntStr(1)+Indx1; %time indx (integer)
timeMaxMinDIC(3,:) = [tMax1, TimeIndx];
StrainMaxMin(3,:) = [Max1, TimeIndx];
scatter(tMax1, Max1, 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on

% 3rd Maximum Strain
tLow = 600;
tHigh = 706;
IntStr = find(t_DIC >=tLow & t_DIC <= tHigh);
[Max1, Indx1] = max(AbsoluteStrain(IntStr));
tMax1 = tLow + t_DIC(Indx1); %time (s)
TimeIndx = IntStr(1)+Indx1; %time indx (integer)
timeMaxMinDIC(5,:) = [tMax1, TimeIndx];
StrainMaxMin(5,:) = [Max1, TimeIndx];
scatter(tMax1, Max1, 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on
```

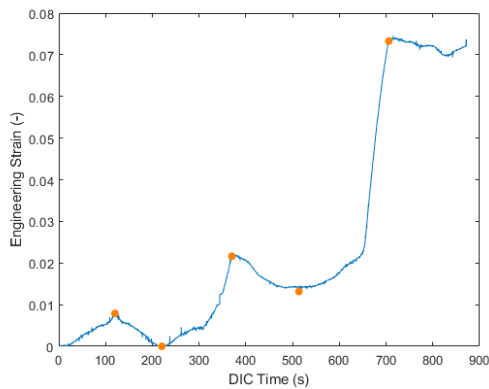


```

% 1st minimum Strain
tLow = 200;
tHigh = 300;
IntStr = find(t_DIC >= tLow & t_DIC <= tHigh);
[Min1, Indx1] = min(AbsoluteStrain(IntStr));
TimeIndx = IntStr(1)+Indx1; % index of minimum in time - serves to couple curve later
tMin1 = tLow + t_DIC(Indx1);
timeMaxMinDIC(2,:) = [tMin1, TimeIndx];
StrainMaxMin(2,:) = [Min1, TimeIndx];
% mstrain(1,:) = [TimeIndx, Min1];
scatter(tMin1, Min1, 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on

% 2nd minimum Strain
tLow = 450;
tHigh = 513.4;
IntStr = find(t_DIC >= tLow & t_DIC <= tHigh);
[Min1, Indx1] = min(AbsoluteStrain(IntStr));
TimeIndx = IntStr(1)+Indx1; % index of minimum in time - serves to couple curve later
tMin1 = tLow + t_DIC(Indx1);
timeMaxMinDIC(4,:) = [tMin1, TimeIndx];
StrainMaxMin(4,:) = [Min1, TimeIndx];
% mstrain(1,:) = [TimeIndx, Min1];
scatter(tMin1, Min1, 'filled', 'MarkerFaceColor',[1 0.5 0]);
hold on

```



Delta clock in TimeMachine and TimeDIC

```

Delta = timeMaxMinDIC(:,1) - TimeMaxMin(:,1);
AvgDelta = mean(Delta(:,1));
t_DIC_original = t_DIC;
t_DIC = t_DIC_original - AvgDelta; % Shifting t_DIC to match TimeMachine

```

COUPLING CURVES for ENGINEERING STRESS - ENGINEERING STRAIN

```

% Engineering Strains for each cycling step (loading and unloading) from
% Step 1 (1st Loading) to Step 5 (3rd Loading - before the final unloading)
EngStrainStep1 = AbsoluteStrain(1:StrainMaxMin(1,2));

```

```

EngStrainStep2 = AbsoluteStrain(StrainMaxMin(1,2):StrainMaxMin(2,2));
EngStrainStep3 = AbsoluteStrain(StrainMaxMin(2,2):StrainMaxMin(3,2));
EngStrainStep4 = AbsoluteStrain(StrainMaxMin(3,2):StrainMaxMin(4,2));
EngStrainStep5 = AbsoluteStrain(StrainMaxMin(4,2):StrainMaxMin(5,2));

% time intervals from the DIC (Step 1 - Step 5)
timeDIC1 = t_DIC(1:StrainMaxMin(1,2));
timeDIC2 = t_DIC(StrainMaxMin(1,2):StrainMaxMin(2,2));
timeDIC3 = t_DIC(StrainMaxMin(2,2):StrainMaxMin(3,2));
timeDIC4 = t_DIC(StrainMaxMin(3,2):StrainMaxMin(4,2));
timeDIC5 = t_DIC(StrainMaxMin(4,2):StrainMaxMin(5,2));

% Time intervals from the Testing Machine (Step 1 - Step 5)
timeM1 = timeMachine(1:TimeMaxMin(1,2));
timeM2 = timeMachine(TimeMaxMin(1,2): TimeMaxMin(2,2));
timeM3 = timeMachine(TimeMaxMin(2,2): TimeMaxMin(3,2));
timeM4 = timeMachine(TimeMaxMin(3,2): TimeMaxMin(4,2));
timeM5 = timeMachine(TimeMaxMin(4,2): TimeMaxMin(5,2));

% Interpolation of Strain over time intervals from Machine (extrapolating data to
match the vector size of EngStress, see below)
EngEpsilon1 = interp1(timeDIC1,EngStrainStep1,timeM1, 'linear', 'extrap');
EngEpsilon2 = interp1(timeDIC2,EngStrainStep2,timeM2, 'linear', 'extrap');
EngEpsilon3 = interp1(timeDIC3,EngStrainStep3,timeM3, 'linear', 'extrap');
EngEpsilon4 = interp1(timeDIC4,EngStrainStep4,timeM4, 'linear', 'extrap');
EngEpsilon5 = interp1(timeDIC5,EngStrainStep5,timeM5); % here 'linear', 'extrap'
makes noise at the toe of the curve

% Checking interpolation allowed correct creation of vectors
SIZEcheck = zeros(5,4);
SIZEcheck(1,:) = [size(EngEpsilon1,1); size(EngStrainStep1,1); size(timeDIC1,1);
size(timeM1,1)];
SIZEcheck(2,:) = [size(EngEpsilon2,1); size(EngStrainStep2,1); size(timeDIC2,1);
size(timeM2,1)];
SIZEcheck(3,:) = [size(EngEpsilon3,1); size(EngStrainStep3,1); size(timeDIC3,1);
size(timeM3,1)];
SIZEcheck(4,:) = [size(EngEpsilon4,1); size(EngStrainStep4,1); size(timeDIC4,1);
size(timeM4,1)];
SIZEcheck(5,:) = [size(EngEpsilon5,1); size(EngStrainStep5,1); size(timeDIC5,1);
size(timeM5,1)];

% Loads relative to Step 1 - Step 5
F1 = StdForce_N(1:TimeMaxMin(1,2));
F2 = StdForce_N(TimeMaxMin(1,2): TimeMaxMin(2,2));
F3 = StdForce_N(TimeMaxMin(2,2): TimeMaxMin(3,2));
F4 = StdForce_N(TimeMaxMin(3,2): TimeMaxMin(4,2));
F5 = StdForce_N(TimeMaxMin(4,2): TimeMaxMin(5,2));

% Engineering Stress (Step 1 - Step 5)
EngStressStep1 = F1./A0;
EngStressStep2 = F2./A0;
EngStressStep3 = F3./A0;
EngStressStep4 = F4./A0;
EngStressStep5 = F5./A0;

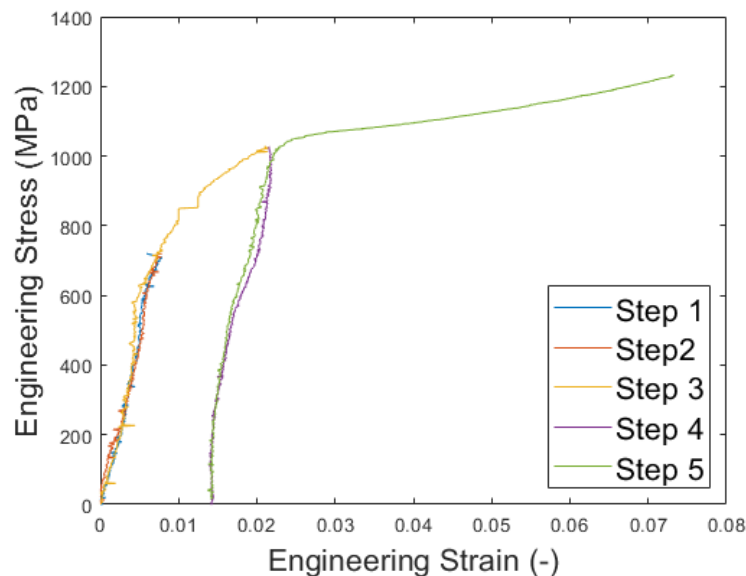
```

```

% Full vectors of Engineering Strain and Engineering Stress to allow
% plotting of the curve
EngEpsilon = [EngEpsilon1; EngEpsilon2; EngEpsilon3; EngEpsilon4; EngEpsilon5];
EngStress = [EngStressStep1; EngStressStep2; EngStressStep3; EngStressStep4;
EngStressStep5];

fig4 = figure('NumberTitle', 'off', 'name', 'Engineering curve - coupled and
interpolated StepbyStep', 'Color', [1 1 1]);
plot(EngEpsilon1, EngStressStep1)
hold on
plot(EngEpsilon2, EngStressStep2)
hold on
plot(EngEpsilon3, EngStressStep3)
hold on
plot(EngEpsilon4, EngStressStep4)
hold on
plot(EngEpsilon5, EngStressStep5)

```



Estimate of Elastic Modulus by Secant Method over the first unloading in the Stress-Strain Curve (Step2)

```

SigmaMax = LoadMaxMin(1,1)/A0; % Max Engineering Stress in Step2
SigmaMin = LoadMaxMin(2,1)/A0; % Min Engineering Stress in Step2

Indx2a = find(EngStressStep2 == SigmaMax);
Indx2b = find(EngStressStep2 == SigmaMin);

EpsilonMax = EngEpsilon2(Indx2a); % Engineering Strain relative to Max Engineering
Stress in Step2
EpsilonMin = EngEpsilon2(Indx2b); % Engineering Strain relative to Min Engineering
Stress in Step2

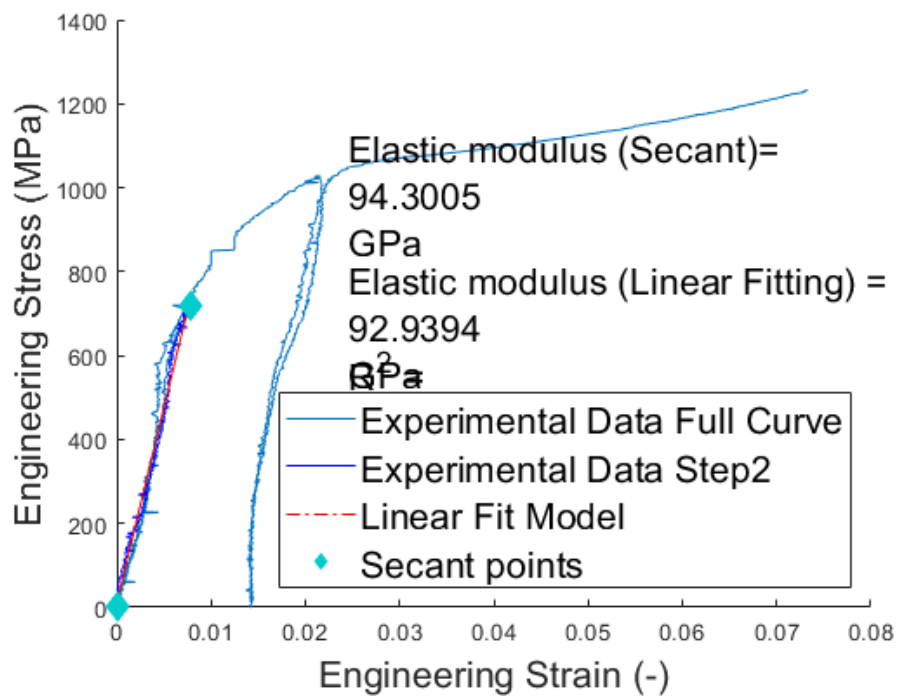
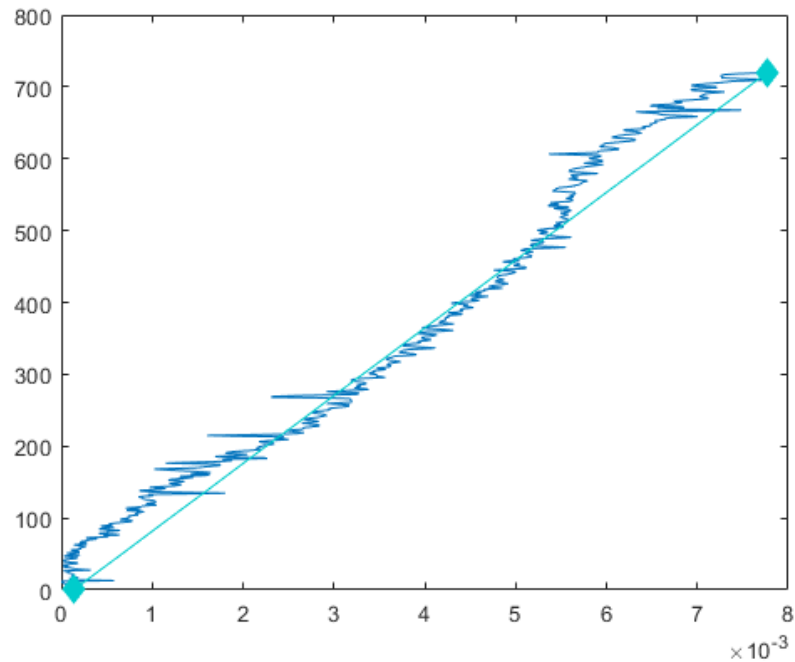
ESecantEngGPa = (SigmaMax - SigmaMin)/((EpsilonMax - EpsilonMin)*1000); % Elastic

```

Modulus by SECANT method (Step2) (GPa)

```
EpsilonSecEng = [EpsilonMax; EpsilonMin];
SigmaSecEng = [SigmaMax; SigmaMin];

figure
plot(EngEpsilon2, EngStressStep2)
hold on
scatter(EpsilonSecEng,SigmaSecEng , (100), 'filled','diamond', 'MarkerFaceColor',[0
.8 .8])
hold on
plot(EpsilonSecEng,SigmaSecEng , 'color',[0 .8 .8])
%
fig5 = figure('NumberTitle', 'off', 'name', 'Engineering curve - interpolated
StepbyStep', 'Color', [1 1 1]);
hold on
% title('Full Compression curve in cyclic loading with Elastic Modulus - Stress vs
Strain Curve interpolated step by step', 'FontSize',24)
xlabel('Engineering Strain (-)', 'FontSize',16)
ylabel('Engineering Stress (MPa)', 'FontSize', 16)
E_Step2Eng = fitlm(EngEpsilon2, EngStressStep2); % linear model
R_squared = E_Step2Eng.Rsquared.Ordinary; % R-squared
E_eng = E_Step2Eng.Coefficients.Estimate(2); %MPa
%q_t = E_Step2Eng.Coefficients.Estimate(1); %intercept q
E_Eng_GPa = E_eng/1000;
plot(EngEpsilon, EngStress)
hold on
plot(EngEpsilon2, E_Step2Eng.Variables.y, 'b-')
hold on
plot(EngEpsilon2, E_Step2Eng.Fitted, 'r-')
hold on
scatter(EpsilonSecEng,SigmaSecEng , (100), 'filled','diamond', 'MarkerFaceColor',[0
.8 .8])
hold on
str = {'Elastic modulus (Linear Fitting) =' E_Eng_GPa 'GPa'};
str2 = {'R^2 =' R_squared};
PosX = min(EngEpsilon)+(max(EngEpsilon)-min(EngEpsilon))/3; % Defining position of
texts in the figure (along the TrueStrain Axis)
PosYE = max(EngStress)-(max(EngStress)-min(EngStress))/2.2; % Defining position of
text for E in the figure (along the TrueStress Axis)
PosYR = max(EngStress)-(max(EngStress)-min(EngStress))/1.7; % Defining position of
text for R-squared in the figure (along the TrueStress Axis)
text(PosX,PosYE,str, 'FontSize',16)
text(PosX,PosYR , str2, 'FontSize',16)
hold on
str3 = {'Elastic modulus (Secant)= ' ESecantEngGPa 'GPa'};
PosX3 = min(EngEpsilon)+(max(EngEpsilon)-min(EngEpsilon))/3;
PosYSEc = max(EngStress)-(max(EngStress)-min(EngStress))/5.0;
text(PosX3,PosYSEc,str3, 'FontSize',16)
legend({'Experimental Data Full Curve', 'Experimental Data Step2','Linear Fit Model',
'Secant points'}, 'Location', 'southeast', 'FontSize',16)
```



Elastic Modulus Secant by using sigma = 400 MPa as upper limit within Step2

```
% Engineering curve
UpperPoint = find(EngStressStep2 >= 399 & EngStressStep2 <= 401);
SigmaEngUpperPoint = EngStressStep2(UpperPoint); % Max Stress. Stress ~400 MPa
EngStrainUpperPoint = EngEpsilon2(UpperPoint); % Strain relative to the Max Stress
above (-)
```

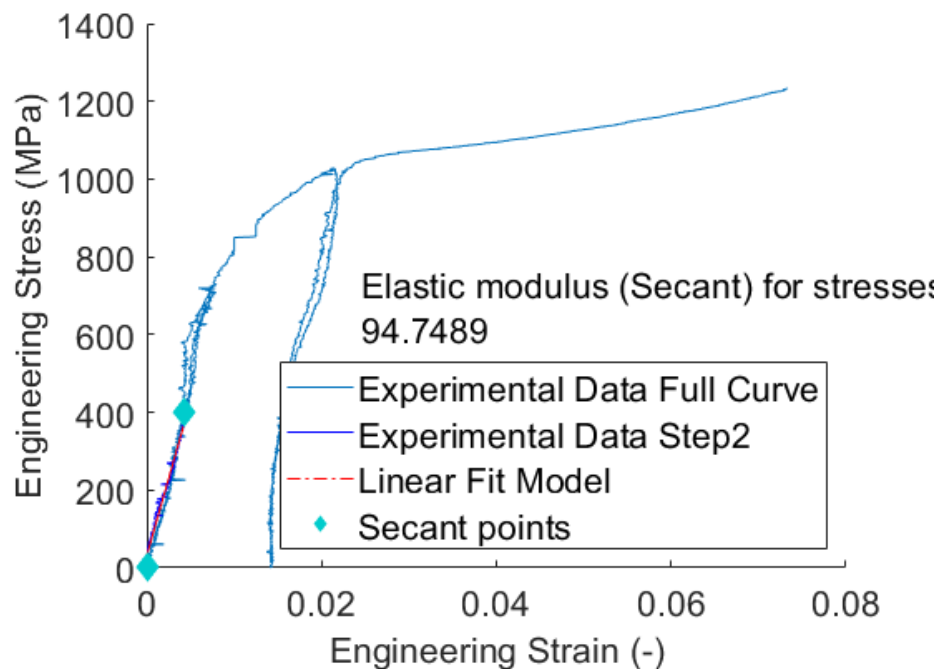
```

E_sec_UpP = (SigmaEngUpperPoint - SigmaMin)/((EngStrainUpperPoint -
EpsilonMin)*1000); % Elastic Modulus evaluated in Step 2 between 0-400 MPa in Stress
ScatterPoints = [EngStrainUpperPoint, SigmaEngUpperPoint; EpsilonMin, SigmaMin];

figure
axes('FontSize', 16);
hold on
plot(EngEpsilon, EngStress)
hold on
plot(EngEpsilon2(UpperPoint:end), E_Step2Eng400.Variables.y, 'b-')
hold on
plot(EngEpsilon2(UpperPoint:end), E_Step2Eng400.Fitted, 'r-.')
hold on
scatter(ScatterPoints(:,1), ScatterPoints(:,2), (100), 'filled','diamond',
'MarkerFaceColor',[0 .8 .8])
hold on

str3 = {'Elastic modulus (Secant) for stresses between 0 MPa and 400 MPa=' E_sec_UpP
'GPa'};
PosX3 = min(EngEpsilon)+(max(EngEpsilon)-min(EngEpsilon))/3;
PosYSEc = max(EngStress)-(max(EngStress)-min(EngStress))/2;
text(PosX3,PosYSEc,str3, 'FontSize',16)
xlabel('Engineering Strain (-)', 'FontSize',16)
ylabel('Engineering Stress (MPa)', 'FontSize',16)
legend({'Experimental Data Full Curve', 'Experimental Data Step2','Linear Fit Model',
'Secant points'}, 'Location', 'southeast', 'FontSize',16)

```



Full Engineering Stress vs Engineering Strain curve

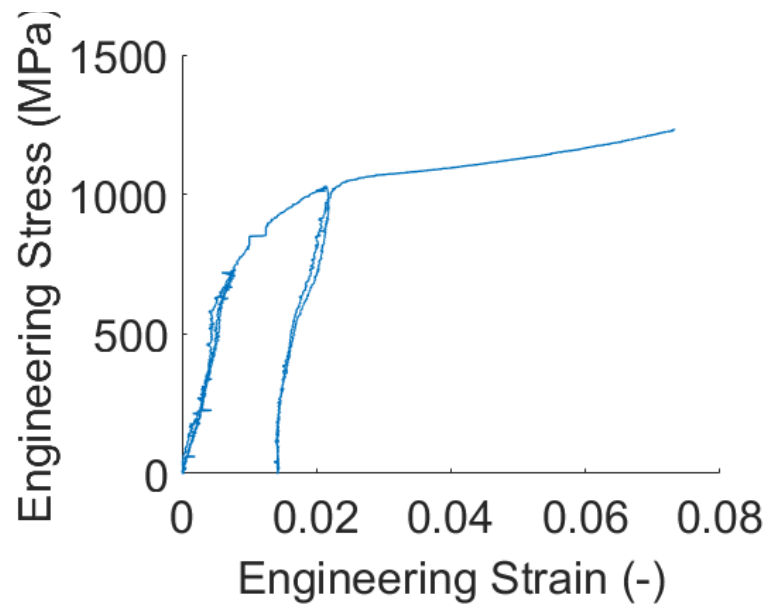
```

figure
axes('FontSize', 24);

```

```
hold on
plot(EngEpsilon, EngStress, 'Linewidth', 1)
hold on

xlabel('Engineering Strain (-)', 'FontSize', 24)
ylabel('Engineering Stress (MPa)', 'FontSize', 24)
```



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Appendix II

Rationale of the biological tests used in this study

This appendix provides the rationale of the chosen biological tests and protocols used in this study. First, it highlights the motivation of the biological tests for zirconium and zirconium lattices, and it outlines the constraints on the biological testing for this research (§A.II.1). It illustrates and discusses the international standards that exist on the biocompatibility assessment (§A.II.2), and the cytotoxicity testing is reviewed in detail (§A.II.3). It presents and discusses the cytocompatibility testing and the protocols available in the literature (§A.II.4), before illustrating the colorimetric assays (§A.II.5) and Live/Dead cell staining methods (§A.II.6). The opportunity to couple colorimetric assays with other biological methods is explored in §A.II.7, and the choice of the osteoblast-like MG-63 cells is discussed in §A.II.8.

A.II.1 Development of biological methods used in this study

One of the objectives of the study was to investigate the biological performance of the L-PBF-fabricated zirconium octet lattice structures zirconium. Indeed, although zirconium has shown good biocompatibility and no adverse biological response has been reported, the literature is still limited and there is *no available work on porous zirconium* (let alone zirconium lattices).

Porous structures expose cells and the biological environment in general to a higher surface area, and this might affect biocompatibility because of the increased and generally different interaction between the material and cells or tissues (ASTM F748-16 [184]). Furthermore, the irregular surfaces and complex topography of the lattice's internal surfaces might affect the cellular behaviour and impact the biocompatibility.

In this study, the methods to assess the biocompatibility of zirconium lattices were selected according to the following criteria:

- 1) They should allow to answer the research question “how do L-PBF-fabricated Zr702 octet lattice structures behave in a biological perspective?”.
- 2) They must be *in vitro*.

In vitro (“in glass”, from Latin) tests are performed “outside a living organism”, in a well-controlled environment (for example, in Petri dish), in contrast with *in vivo* (“within a living organism”, from Latin) studies, which require experimentation on animals or clinical trials. For this work, *in vitro* methods were preferred over the *in vivo* ones for three main reasons.

First, *in vitro* studies are indicated for preliminary screenings, which are appropriate in this case, as the knowledge on biocompatibility of zirconium is still limited and no information on porous zirconium is available. Indeed, *in vitro* tests should be performed and reviewed before *in vivo* studies. These tests can provide invaluable information in early stages and save animals (or humans) from unnecessary sufferance (or harm, for humans) [184, 185].

Second, these tests are relatively fast and inexpensive compared to *in vivo* studies (ASTM F748-16 [184]). Also, they do not require particularly lengthy or special training, and can be mastered by people with an engineering background, provided they have experience in working under sterile conditions (which was the case for the author of this thesis).

Third, they can be performed without going through any ethical approval processes (*in vivo* studies need to be approved before being carried out, instead). These procedures are time-consuming, and - given the duration of the procedures - they were not compatible with the timeframe for this research study.

A.II.2 Biocompatibility assessment guidelines for orthopaedic devices by international organisations

Before moving to review the literature that helped develop the biological methods used in this work, it is worth introducing the norms relative to the biocompatibility testing, as this helps in the understanding of the full process that allows to determine whether materials and medical devices are biocompatible, hence safe to use in the sought application.

The main guidelines on biocompatibility testing are provided by the ISO 10993-1:2020 [185] and the ASTM F748-16 [184].

As mentioned in the rationale of the ASTM guideline [184], the ISO 10993-1:2020 [185] was developed following the ASTM F748-16 [184]. As such, these norms [184, 185] work within the same framework and under the same principles, and ultimately recommend the same type of tests. Hence, the two guidelines [184, 185] will be discussed together.

It is worth highlighting that the biocompatibility assessment (of a material and/or a medical device) is not performed in a single assay, but is rather composed of several endpoints, the choice of which depends – among others – on the information already available and the expected application of the material/device.

In the case of permanent¹⁶ implant devices that are in contact with bones (like orthopaedic prostheses), biocompatibility can be evaluated against the biological effects listed in Table 31. Of these, cytotoxicity and genotoxicity tests are the only *in vitro* tests. Cytotoxicity tests are used and preferred [186] in early screening studies to investigate material biocompatibility and are widely exploited to test newly developed materials, from academia to industry [187]. Instead, genotoxicity testing is rarely adopted by researchers in Engineering¹⁷ Schools who deal with metallic materials for biomedical applications.

¹⁶ Permanent medical devices are in contact with the body for a period of time longer than thirty days, according to the ISO 10993-1:2020 185. *BS EN ISO 10993-1:2020: Biological evaluation of medical devices: Evaluation and testing within a risk management process*. 2020, British Standards Institute.

¹⁷ The author appreciates that this might not be the case for scientific research in general, and that this observation is limited only to the specific niche in which the author is working.

Table 31 Biocompatibility assessment endpoints recommended by ISO 10993-1:2020 [185].

Biological effect	Objective of the test	Test class
Cytotoxicity	Determining cell death, inhibition of cell growth, colony formation, and other effects	<i>In vitro</i>
Sensitization	Assess the potential for contact sensitization by materials, medical devices or their extracts	<i>In vivo</i>
Irritation or Intracutaneous Reactivity	Assess the irritation potential of materials, medical devices or their extracts	<i>In vivo</i>
Acute System Toxicity	Estimate potential harmful effects of materials or medical devices for contact duration <24 h	<i>In vivo</i>
Subacute Toxicity	Estimate potential harmful effects of materials or medical devices for contact duration ≥24 h and up to a period ≤ 10% of	<i>In vivo</i>
Subchronic Toxicity	lifespan of the test animal	<i>In vivo</i>
Chronic Toxicity	Estimate potential harmful effects of materials or medical devices for contact duration ≥ 10% of lifespan of the test animal	<i>In vivo</i>
Pyrogenicity	Detect material-mediated pyrogenicity	<i>In vivo</i>
Genotoxicity	Assess the potential for DNA or gene toxicities	<i>In vitro</i> , potentially followed by <i>in vivo</i> tests
Implantation effects	Assess the local pathological effects on living tissue	<i>In vivo</i>
Reproductive and developmental toxicity	Assess the potential effects of materials or medical devices on reproductive function, teratogenicity, and prenatal and early postnatal development	<i>In vivo</i>
Carcinogenicity	Assess the potential cancerogenic effects of materials and devices	<i>In vivo</i>

Because zirconium is not suspected to cause any gene mutations and in fact is considered bio-inert, genotoxicity tests were excluded from the candidate methods in this study, following the recommendations of ISO 10993-1:2020.

A.II.3 Cytotoxicity testing according to ISO 10993-5:2009

As briefly outlined in Table 31, cytotoxicity testing aims at assessing the potential toxic effect of a material/medical device on cultured cells.

According to ISO 10993-5:2009 [188], cytotoxicity can be evaluated by morphological analysis of cells, cell damage or cell growth measurements, or by measuring specific aspect of cellular metabolism. ISO 10993-5:2009 [188] contemplates three classes of cytotoxicity tests: extract, direct contact, and indirect contact tests.

The extract tests work with extracted substances of the material under investigation, and the solution (extract) is used as cell culture medium¹⁸ onto cultured cell monolayers to mimic the effect of the material on biological tissues. The cytotoxicity is then evaluated based on the cell morphology, membrane integrity, or amount of living cells after specific times from the introduction of the extract [189]. The latter is typically assessed by colorimetric analyses, which will be discussed in more detail in the following section (§A.II.5).

Regarding direct contact tests, cultured cell monolayers are put in contact with the test material for specific times, and the material cytotoxicity is evaluated based on the cell morphology and number of viable cells. For example, Ansar *et al.* [59] estimated the cell viability by MTT assay (a colorimetric test which is described in detail in §A.II.5), to assess the cytocompatibility of chemically-treated titanium disks.

Finally, indirect contact methods encompass a variety of tests, in which cell monolayers are separated from the material under investigation by a medium (for example, agar layer or filter), so that the leachable substances coming from the samples of interest are tested. In these tests, the morphology and/or the membrane integrity are analysed to determine the cytotoxicity/cytocompatibility of a material [189].

¹⁸ Cell culture *medium* (pl. *media*) is a liquid or gel that supports cell growth and provides the nutrients for cells for *in vitro* studies.

Cytotoxicity testing according to ISO 10993-5 [188] is fast, simple, offers a high sensitivity [186], and is the method of choice in several research works ([190],[22], [36] just to cite a few).

However, the ASTM highlights that such tests are not universally recognised as a good method to proof biocompatibility of materials, as some non-biocompatible materials may pass this test, and some others would be excluded albeit being biocompatible [184].

More importantly, these tests only assess the cellular damage exerted by the leachables (soluble or insoluble) of the material [187, 189]. Indeed, cells are cultured on common *in vitro* substrates (tissue-culture plastic) until confluence is reached, and no protocol that would involve the direct cell adhesion onto the tested material is prescribed. For their nature, such tests are not designed to assess the biological effects due to an early interaction between cells and the biomaterial.

A.II.4 From cytotoxicity to cytocompatibility testing

Because cytotoxicity testing as per ISO 10993-5 [188] would not allow to assess the cell behaviour (adhesion, spreading, growth, etc.) in direct contact with zirconium bulk and lattices, the author searched for other protocols that have already been used in the literature, with a particular focus on studies that dealt with porous metallic substrates.

The methods that were finally considered are classed as “cytocompatibility” tests. These allow to evaluate the cellular response to materials in a wider perspective compared to the cytotoxic tests [189], and they are aimed at assessing the structural and functional characteristics of cells exposed to materials and medical devices [189]. In particular, these tests evaluate the cell adhesion, viability, proliferation, cell cycle, and differentiation (in the case of stem cells) on the scaffolds/materials of interest.

Although such protocols are not enclosed in ISO or ASTM guidelines and norms, cytocompatible materials are considered non-cytotoxic [189]. Hence, proving cytocompatibility is equivalent to prove the absence of cytotoxic effects.

Among the others, cell viability tests are those who allow to address the questions related to the biological performance of the materials and scaffolds (lattices) investigated here.

Two approaches are commonly exploited to evaluate the cell viability for cytocompatibility assessment: 1) Live and Dead Staining; 2) colorimetric tests, which were a prescribed test also in the ISO 10993-5 [188] (refer to §A.I.3).

Table 32 summarises the working principles of the methods and reports the studies analysed that exploited such techniques. A detailed analysis of the methods is provided in the next sections §A.I.5 and §A.I.6.

Table 32 Cell viability techniques used in literature to demonstrate the material biocompatibility.

Technique	Working principle and examples	References
Live/Dead Cell Staining	Qualitative and quantitative analysis in which one or two markers are added to the cell culture to stain selectively live and/or dead cells. Examples include Trypan blue, Neutral red, and fluorescent dyes (Calcein acetoxymethyl/ethidium homodimer, Propidium Iodide, etc.).	Aristizabal <i>et al.</i> [7]
Colorimetric analysis	Quantitative analysis that allows to estimate the number of viable cells in a system from a measured absorbance. Complete and detailed mechanism is given in §A.I.5. Examples include MTT assay, WST-1 assay, CKK-8 assay, etc.	Varkey <i>et al.</i> [174] Jonitz <i>et al.</i> [175]

A.II.5 The colorimetric analyses: a way to estimate the cell viability on porous biomaterials

Colorimetric analyses for cell viability are quantitative analyses that exploit biochemical reactions to estimate indirectly the number of viable cells in a system for *in vitro* studies. Strictly speaking, such assays evaluate the cellular metabolic activity: the reagent introduced into cell cultures is a tetrazolium salt – yellow in colour- is reduced (or “digested”) by highly metabolically active cells to produce formazan crystals, purple in colour (the chemical reaction is reported in Figure 61a). As the reaction tetrazolium salt-formazan does not occur within dead cells, the amount of produced formazan is

proportional to the quantity of viable cells, enabling an indirect estimation of the number of viable cells in a system [174].

The working principle of these colorimetric assays can be better understood by observing the photograph of a well plate containing a set of formazan solutions in Figure 61a. As the wells were seeded with (known and) increasing number of cells from the left to the right, the solutions' colours gradually intensify, from faint pink (far-left in the well-plate) to dark purple (far-right).

The colorimetric assay is a quantitative method, as the absorbance of formazan-containing solutions is measured. Calibration curves are commonly derived to relate the measured absorbance to the number of viable cells (indeed, the photograph in Figure 61a represents a calibration experiment carried out by the author of this thesis).

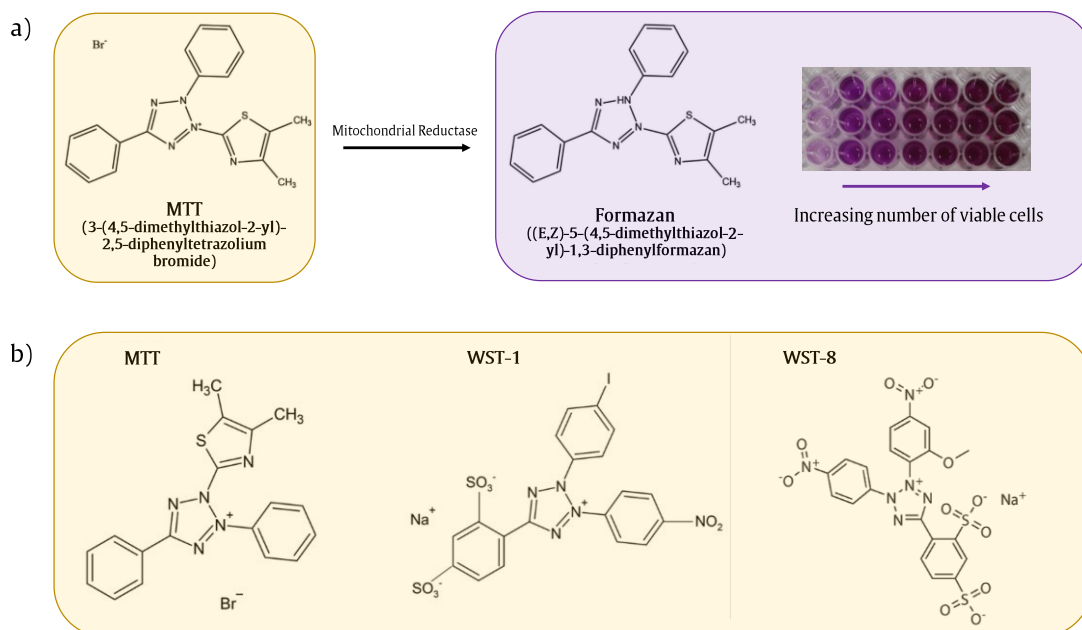


Figure 61 a) Chemical reaction and working principle of the colorimetric assays for cell viability (MTT assay); b) Molecular structure of the most common tetrazolium salts used in colorimetric tests for cell viability (MTT, WST-1, and WST-8, the latter of which is used in the CCK-8 assay).

Several assays exist: among the others, MTT assay, WST-1 assay and the cell counting kit “CCK-8” are commonly used. They all exploit the reaction illustrated in Figure 61a (in which the MTT is showcased), however differ for the specific reagent molecule that is used. In fact, these techniques (MTT, WST-1, etc.) are generally named after the particular

tetrazolium salt used. The chemical structure of the reagents MTT, WST-1, and WST-8 (the reagent used in the CCK-8) are shown in Figure 61b.

As the execution of colorimetric tests for cell viability is not normed for porous substrates such as lattice structures and poses some challenges, several protocols have been developed and are found in the literature.

Liang *et al.* [97] cultured MG-63 cells on L-PBF-fabricated Ti6Al4V porous disks, and attempted to measure the absorbance of cell suspensions with the CCK-8 kit. Interestingly, the cells were detached from the scaffolds, at specific times (1, 3 and 5 days), before performing the assay. Only later, the CCK-8 reagent (WST-8) was left to be digested by the living cells. This protocol looked interesting, as it promised to reveal the cell proliferation that would have occurred onto the scaffolds. Nevertheless, the authors reported that the measurement of the absorbance of a blank without clarifying which substance it was used. Even more importantly, it appears that the absorbance of the *blank* steadily increased with the culture time (see Figure 62). As such, it is not clear whether the increased absorbance of the test cell solutions (collected from the seeded substrates under investigation) over time was due to the varying absorbance of the liquid (the blank), or to the scaffold.

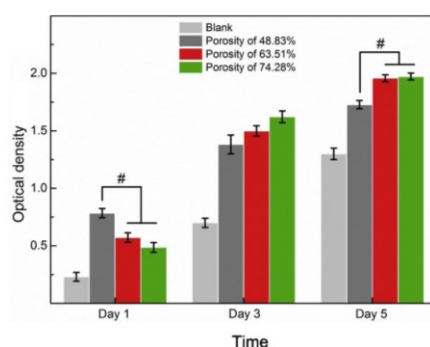


Figure 62 Colorimetric assays methods and results performed on porous scaffolds for *in vitro* studies. (d) illustrates the results reported by Liang *et al.* [97], in which the lack of clarity on the blank used impedes to replicate their experiments. Image from [97].

Unfortunately, the lack of the information on this part of the protocol hinders the replication of the study. Furthermore, it impedes to correctly interpret the data, to

compare them with similar studies, and to fully understand the implications of the experimental results.

In contrast with this approach are the protocols used by Varkey *et al.* [174] and of Jonitz *et al.* [175]. Although some differences in their methods exist (see Table 33), the approach is the same: instead of detaching the cells from the scaffolds and performing the colorimetric test on cell suspensions, the tetrazolium salt was added directly onto the scaffold of interest. This allows to measure the metabolic activity of the osteoblasts attached to the substrates (rather than in a suspension) and to estimate the cell viability when the cells are in direct contact with the material of interest. These methods are rather simple and fast, and can be used to infer on the cell viability on porous materials.

Table 33 Main differences between the colorimetric test methods used by Varkey et al. [174] and Jonitz et al. [175]

Study	Colorimetric test	Cell type used	Time of interest (days)	Removal of liquids	Control	Substrate
Varkey <i>et al.</i> [174]	MTT assay	MG-63	4	yes	Tissue-culture plastic	Silk fibroin scaffolds
Jonitz <i>et al.</i> [175]	WST-1 assay	Human primary osteoblasts	1 and 8	no	Medium	3D printed Tricalcium-phosphate

At observation times of interest, the reagent of the colorimetric assay was added to the cell culture, allowing the “reaction/digestion” to take place. At the end of the incubation time, Varkey *et al.* [174] withdrew the liquids to leave the formazan crystals on the scaffolds undisturbed. The formazan was then dissolved in hydrochloric acid and aliquots of the solutions were analysed using a microplate reader. In contrast with this, Jonitz *et al.* [175] withdrew the solution containing the formazan. This difference in the protocol is due to the different tetrazolium salt used: whilst MTT (used by [174]) leads to water-insoluble formazan, WST-1 (used in Jonitz *et al.* [175]) is digested to a formazan that can

be dissolved well in the reagent-and-media solution by shaking the plate¹⁹. Albeit faster, WST-1 assay requires to use the medium as a control, as its colour affects the absorbance of the formazan-containing solution.

The MTT assay is a cheap, relatively fast, and easy colorimetric analysis. It is a well-established test, and is known for its sensitivity, as it can detect as low as two hundreds living cells [191]. Therefore, the MTT assay was selected for this study to estimate the cell viability on the different substrates.

A.II.6 Live and Dead cell staining: assessing cell viability, morphology and location on biomaterials

Live and Dead cell staining enables to discriminate between viable and dead cells that are attached to substrates and scaffolds. Live and Dead cell staining allows not only to evaluate cell viability, but also cell morphology, location and distribution over a given substrate.

Given that the study on L-PBF-manufactured Zirconium by Aristizabal *et al.* [7] - which constitutes the main foundation of this work - had investigated the biocompatibility of the material by live/dead cell staining, the author chose to assess the same material (in bulk and lattice form) with the same technique.

Several assays for Live/Dead exist, as already shown in Table 32 Cell viability techniques used in literature to demonstrate the material biocompatibility. Here, the live/dead staining with Calcein-AM and Propidium Iodide is presented, as these were used in the work of Aristizabal *et al.* [7].

Calcein-AM is a fluorescent dye that penetrates both dead and live cells, however it is only retained by viable cells. Indeed, Calcein-AM is used to make viable cells visible (with a green fluorescence) under a fluorescence microscope. Propidium Iodide, instead, is impermeable to living cells, it binds to the cell's DNA and emits red fluorescence [189, 192].

¹⁹ WST-1 is a water-soluble salt and was developed precisely to avoid the solubilization step to be performed with different solvents 191. Ligasová, A. and K. Koberna, *DNA Dyes-Highly Sensitive Reporters of Cell Quantification: Comparison with Other Cell Quantification Methods*. Molecules, 2021. 26(18): p. 5515..

A.II.7 Coupling colorimetric test with other methods: a way to bullet-proof biological studies?

Biological testing is - unfortunately - problematic when it comes to experiments repeatability. This issue is well known across the bioengineering community, and it is precisely for this reason that biological studies require an elevated sample size.

Beside the sample size, another way to control the quality of biological experiments is to perform two (or more) complementary tests to assess whether the results of independent measurements are coherent. This approach has already been exploited by several researchers. Here, the author limits to list the work of those who focussed on the colorimetric assays to estimate the cell viability. Jamshidi *et al.* [138] coupled Alamar Blue assay with SEM microscopy, whilst Balla *et al.* [96] and Varkey *et al.* exploited the MTT assay with SEM microscopy to estimate the cell viability and to observe the cell growth and morphology on the samples. In a similar way, Ran *et al.* [193] coupled the CCK-8 assay and SEM microscopy, whilst Liang *et al.* [97] added to these tests the Alkaline Phosphatase Assay Kit, to measure the ALP activity. Finally, Cheng *et al.* [22] and Jonitz *et al.* [175] performed the Kit Live/Dead as well as the MTT assay (as per ISO 10993-5 in [22], whilst a special protocol was used in [175]).

In conclusion, MTT assay and Live/Dead fluorescence test were selected in this study with the aim of coupling two types of information: 1) the cellular metabolic activity as an indirect cell viability analysis, and 2) the cell behaviour in terms of cell viability, adhesion, spread, proliferation and morphology. This approach allowed to validate the results of the analysis on cell viability from the MTT assay, as the response is assessed in both the techniques.

A.II.8 The choice of the cell line (MG-63)

The cell line used in this study is the MG-63. These cells are osteoblast-like cells, and they derive from a White, 14-year-old male patient with osteosarcoma [194].

The cell line has been extensively used in tissue-engineering studies, including the works of Varkey *et al.* [174], Nishimoto *et al.* [127], Liang *et al.* [97], Cheng *et al.* [22], and in works that are aimed at investigating the cell behaviour in response to implant's surface topographies [125, 137].

As such, the MG-63 cell line appeared to be the perfect candidate for this study.

A.II.9 Statistical analysis of MTT Assay results

The MTT experimental results were analysed in Minitab software. The choice of the statistical test was taken based on the nature of the experimental results.

Two-way ANOVA is a statistical test that enables the evaluation of two factors at a time as well as their interaction. As in our MTT test substrates and time of cell culture were varied, this statistical test would be used, provided that the model's assumptions were verified. These assumptions are: 1) normal distribution of the observations within each group; 2) homoscedasticity (equal variances among groups). Whilst ANOVA proves to be robust against deviations from normal distributions, this model is not robust against heteroscedasticity. Such a violation leads to Type I error, meaning that non-significant differences between groups would be wrongly interpreted as significant ones.

If homoscedasticity condition is violated, Welch's ANOVA and Games-Howell Post-Hoc test would be performed in the comparisons between groups. Welch's ANOVA is a non-parametric statistical test that can be seen as an extension of the classic one-way ANOVA and is robust under heteroscedasticity conditions by design [195]. With this statistical test, only one factor can be evaluated at a time, hence the interaction between the two factors varied in the experiments cannot be assessed. Nevertheless, Welch's ANOVA enables to determine whether there is statistical difference between groups' means when a factor is varied (at a chosen confidence level). If a statistical difference is found, a post-Hoc test is

needed to determine which means are statistically different. The Games-Howell test is a non-parametric rank-based post-Hoc test that does *not* assume: 1) normality; 2) equal variances (homoscedasticity); 3) equal sample size. Although it is more liberal (or more prone to Type I errors) than other statistical tests, it can work with sample size as small as $n=6$.

In order to verify the ANOVA's model assumptions, descriptive statistic and statistical tests were performed, as described in Table 34. Levene's test is performed to test the homoscedasticity if the sample size of each group is greater than 20. If this condition is not met, the homoscedasticity condition is excluded *a priori*.

Table 34 Verification methods of ANOVA's model assumptions

Property to be verified	Method	Working principle
Homoscedasticity	Levene's test (if $n > 20$ in each group)	H0 (null hypothesis): equal variances between groups; H1 (alternative hypothesis): at least one variance is different
Normality	Kurtosis and Skewness	Skewness and Kurtosis are descriptive statistical values that summarise the lack of symmetry (from a normal distribution) and the peakiness of a distribution, respectively [196]. In general, both Skewness and Kurtosis within $[-2; +2]$ are acceptable for normal distributions.

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